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4-Carboxybenzophenone-Sensitized Photooxidation of Sulfur-Containing Amino Acids. Nanosecond Laser Flash Photolysis and Pulse Radiolysis Studies

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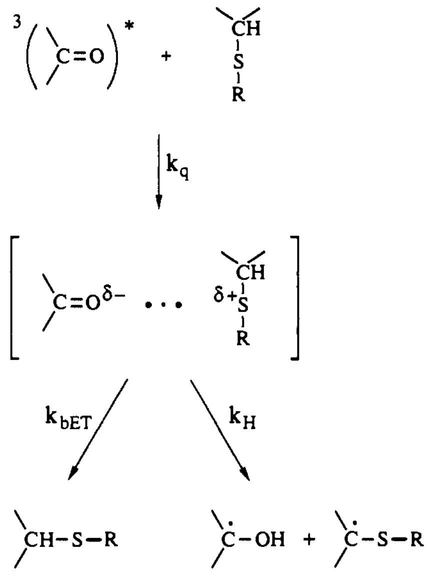
Abstract: Sulfur-containing amino acids were oxidized via photosensitization by 4-carboxybenzophenone (CB) in neutral aqueous solutions. The mechanism of this reaction was investigated with flash photolysis and pulse radiolysis techniques. The rate constants were determined for the quenching of the CB triplet state by 12 amino acids (with variable relative location and number of terminal functions COOH and NH_2 with respect to the sulfur atom) and were found to be 1.8×10^8–2.9×10^9 M^{-1} s^{-1}. Time-resolved transient spectra accompanying the quenching events were assigned to the triplet state, the ketyl radical of CB, the radical anion of CB, and the $(S\therefore S)^+$ radical cations of some of the amino acids. The presence of the radical ions showed the nature of the quenching process to be electron-transfer in character. Two temporally distinct processes were observed for ketyl radical formation. A fast component occurred on a nanosecond time scale. It is ascribed to electron transfer from the sulfur atom to the triplet state of CB followed by (1) the diffusion apart of the charge-transfer (CT) complex and (2) the intramolecular proton transfer within CT complex. The first process was the more efficient one and led to the formation of sulfur-centered radical cations and ketyl radical anions which undergo fast protonation. A slower formation of ketyl radicals occurred on a microsecond time scale and is characterized by pseudo-first-order rate constants, which depend linearly on the CB ground-state concentration ($k \sim 10^9$ M^{-1} s^{-1}). This dark reaction is assigned to the one-electron reduction of CB by the α-aminoalkyl radicals produced from the free-radical cation of the amino acids as a result of intramolecular electron transfer from the carboxyl group to the sulfur-centered radical cation followed by decarboxylation. Yields were determined for some of the processes: the limiting overall quantum yields of ketyl radical (0.4–1.4), the quantum yields of ketyl radical formed in the primary photochemical process and in the dark reaction, and quantum yields of ketyl radical anion. A detailed mechanism for the CB-sensitized photooxidation of sulfur-containing amino acids is proposed and discussed.

Introduction

Electron- and hydrogen-transfer processes in the quenching of aromatic carbonyl triplets by a large variety of organic substrates have been the subject of many photochemical investigations.[1-4] There have also been several studies concerning photochemical generation of radical centers in amino acids,[5-8] peptides,[9] and proteins.[10,11] These two sets of studies come together in biological systems since excited carbonyl triplets can be produced "in vivo",[12,13] either by direct light absorption or by a variety of dark processes, both enzymatically[14] and nonenzymatically.[15] Owing to their electrophilic character, the $n\pi^*$ triplet states of aromatic ketones can be used as typical one-electron oxidants.[2,3] The similarity between the behavior of $n\pi^*$ carbonyl triplets and oxygenated radicals is well-known,[16] and the analogy makes this excited state a suitable model for radical reactions that result in the damage of cell components. To the best of our knowledge, there has not been a systematic, quantitative study that gives a full understanding of the nature of the photooxidation mechanism of the reactions of $n\pi^*$ carbonyl triplets with this interesting class of biological compounds.

Scheme I



Moreover, in the past few years, a growing interest in oxidation processes in biological systems has stimulated several investigations of sulfur-containing amino acids.[5,6,8,17] Thioether groups located in the side chain of these amino acids participate in the maintenance of the structure of protein molecules, form coordination bonds with metal ions, are part of substrate binding sites, and also take part in the transfer of electrons.[18] The nucleophilic sulfur atom is very susceptible to oxidation. Therefore sulfur-centered radicals are expected to play a key role in the migration of unpaired electrons over long distances through the peptide and protein matrix.[19] Considering all the information gathered so far, the oxidation mechanism of sulfur-containing amino acids and peptides has revealed a very important irreversible route, namely, decarboxylation.[20-23] The immediate chemical consequence of decarboxylation is the formation of α-aminoalkyl radicals, which are known to be strongly reducing species.[24] Consequently the damage caused by carbonyl triplets in such biological units may

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appear at positions different from the initial site of attack. This may result in a change of the redox properties of the system and/or in the loss of enzymatic activity accompanied by a change of the native conformation of the protein.

It is thus of great interest to know where and via which mechanism carbonyl triplets preferentially react at specific sites of a sulfur-containing amino acid, and how such reactions are influenced by the local chemical environment of the amino- and carboxyl functional groups. In this paper we shall address some of these questions with respect to the mechanism proposed by Inbar et al.[25,26] and by Cohen and Ojanpera[8] for the quenching of excited triplet states of benzophenone by thioether and by methionine, respectively. According to this mechanism (Scheme I), the initial step is the formation of a charge-transfer complex (CTC) between benzophenone triplet and thioether (k_q). This is followed either by formation of ketyl and α-(alkylthio)alkyl radicals as a result of hydrogen transfer from the carbon (α to a sulfur atom) to the carbonyl oxygen (k_H) or by spin inversion and back electron transfer to regenerate the reactants (k_{bET}). This mechanism has generally been accepted, and it satisfactorily explains some of the experimental observations (high k_q, 10^7–10^9 M^{-1} s^{-1}, generally higher in acetonitrile than in benzene, indicating a polar contribution to the mechanism). Low quantum yields (0.05–0.2) for photoreduction of benzophenone by thioethers are explained in terms of favorable competition between the hydrogen transfer (k_H) and the reversion to the ground state (k_{bET}). For methionine two CT complexes have been proposed:[8] CTC–S [>C–O$^{-}\cdots{}^{+}$S<] and CTC–N [>C–O$^{-}\cdots{}^{+\bullet}$N≤]. These two forms exist in equilibrium, and only CTC–N may lose CO_2 directly to form α-aminoalkyl radicals. It is of significance to note that no evidence for the formation of sulfur-centered radicals was found in these systems.

In this paper we present the results of nanosecond laser flash photolysis studies of the benzophenone-sensitized photooxidation of sulfur-containing amino acids in aqueous solutions. These amino acids have different numbers of the terminal functions, COOH and NH_2, and have varying relative locations of these groups with respect to the sulfur atom. The aim of this work is not only to describe the kinetic aspects of electron transfer to the excited triplet and ground states of 4-carboxybenzophenone, but also to present data regarding transient absorption spectra of various intermediates, including an intermolecular $\text{S}\therefore\text{S}$-bonded radical cation and a ketyl radical anion. The presence of these species is direct proof of dissociation of the CTC–S (involving transfer of a full unit of charge) into separated ions (a process not included either in the Inbar et al.[25] mechanism for sulfides or in the Cohen and Ojanpera[8] mechanism for methionine). Over a longer time scale (microseconds), a second process was observed for ketyl radical generation. This process is ascribed to the one-electron reduction of the benzophenone ground state by the α-aminoalkyl radicals that were formed earlier as a result of an intramolecular electron transfer from the carboxyl group to the sulfur-centered radical cation followed by decarboxylation. Further evidence to support the proposed reaction mechanism of photoinduced electron transfer to the triplet state by sulfur-containing amino acids is provided by the generation of the appropriate α-aminoalkyl-type radicals and kinetic studies in complementary pulse radiolysis experiments. On the basis of the above observations, a modified reaction scheme (Scheme II, vide infra) has been proposed for the photoreduction of benzophenone by sulfur-containing amino acids in aqueous solutions.

Experimental Section

Materials. 4-Carboxybenzophenone (Aldrich) and the sulfur-containing amino acids were obtained from Sigma as the best available grades and were used without further purification. A sample of homomethionine was a generous gift from Professor K.-D. Asmus (HMI, Germany). Water was purified by a Millipore Milli-Q system.

Solutions. The concentrations of sulfur-containing amino acids in the laser flash photolysis experiments were in the range 8×10^{-5}–10^{-3} M (in the quenching experiments) and 2×10^{-2} M (when recording spectra in the time range following full quenching of the 4-carboxybenzophenone triplet). 4-Carboxybenzophenone concentration of 2×10^{-3} M in water was employed in quenching experiments. During monitoring of the secondary process, its concentration was in the range of 2.5×10^{-4}–2×10^{-3} M. All solutions were deoxygenated by bubbling high-purity argon through them. They were buffered in the presence of NaH_2PO_4/Na_2HPO_4 (0.025 M). Pulse radiolysis experiments were performed in order to measure rate constants of the reaction of α-aminoalkyl radicals (derived from various sulfur-containing amino acids) with 4-carboxybenzophenone. Solutions were generally prepared with $\approx 2 \times 10^{-2}$ M sulfur-containing amino acid and with four to six concentrations of 4-carboxybenzophenone ranging from 2×10^{-4} to 2×10^{-3} M. Subsequently, all solutions were saturated with N_2O, in order to convert hydrated electrons into hydroxyl radicals via $e_{aq}^{-} + N_2O \rightarrow {}^{\bullet}OH + OH^{-} + N_2$.

Laser Flash Photolysis. The nanosecond laser flash photolysis apparatus has been described in detail elsewhere[27] with the exception that the computer analysis was in this case performed on a VAX-11/780. Laser excitation at 337.1 nm (≈1–3 mJ, pulse width ≈ 8 ns) from a Molectron UV-400 nitrogen laser was used in a right-angle geometry. Rectangular quartz cells (0.5 × 1 cm) with a path length of 0.5 cm for the monitoring beam were used. The transient absorbances at preselected wavelengths were monitored by a detection system consisting of a monochromator, a photomultiplier tube (PMT), and a 1-kW pulsed xenon lamp as the monitoring source. The signal from the PMT was processed by a 7912 AD Tektronix transient digitizer controlled by an LSI 11/2 microcomputer. A typical experiment consisted of signal-averaging a series of 5–15 decay traces from reproducible shots from the laser. Cutoff filters were used to avoid spurious response to second-order scattering from the monochromator grating.

Pulse Radiolysis. All pulse radiolysis experiments were performed by applying 10-ns pulses of high-energy electrons from the Notre Dame 7 MeV ARCO LP-7 linear accelerator. Absorbed doses were on the order of 4–6 Gy (1 Gy = 1 J/kg). A description of the pulse radiolysis setup and data collection system is reported elsewhere.[28,29] The experiments were carried out with a continuous flow of the sample solution.

Results

1. Quenching of the CB Triplet by Sulfur-Containing Amino Acids. Quenching Rate Constants and Ketyl Radical Quantum Yields.

Twelve sulfur-containing amino acids were used as quenchers of the 4-carboxybenzophenone, CB, triplet state in aqueous solution at pH = 6.8. Since only neutral solutions were studied, it will be understood that the CB triplet is always in its carboxylate anion form[30] (pK_a = 4.5 for CB)[31] and that the amino

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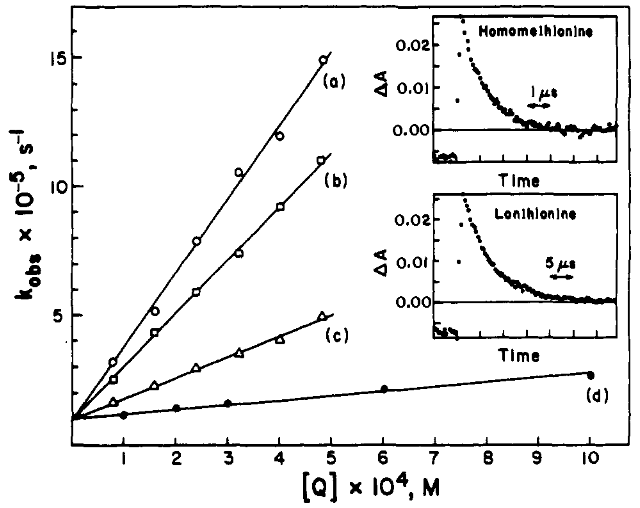

Figure 1. Plots according to eq 1 for 4-carboxybenzophenone (CB) triplet quenching by (a) homomethionine, (b) thiaproline, (c) S-(carboxymethyl)cysteine, and (d) lanthionine in aqueous solution at pH = 6.8. Inserts: experimental traces for CB triplet decay at 540 nm in the presence of 0.32 mM homomethionine (upper insert) and 0.30 mM lanthionine (lower insert).

acids are in their zwitterionic form[32] with the exception of *N*-acetylmethionine, *S*-(carboxymethyl)cysteine, and *S*-(2-carboxyethyl)cysteine. The quenching rate constants, k_q, were obtained from the experimentally measured, pseudo-first-order rate constants, k_{obs}, for the decay of the CB triplet by the formula

$$k_{obs} = \tau_T^{-1} + k_q[Q] \qquad (1)$$

where τ_T is the lifetime of the CB triplet in the absence of a quencher.

Typical experimental traces for triplet decay in the presence of about 0.3 × 10^{-3} M homomethionine and lanthionine are presented in the insets of Figure 1. The long-lived absorption has been attributed to the formation of the 4-carboxybenzophenone ketyl radical, K, that was established by comparison of the observed transient spectra with that from Inbar et al.[30] (absorption maximum at 570 nm). Whenever the efficiency of ketyl formation was low, e.g. homomethionine, the triplets produced by flash excitation were monitored at their long-wavelength absorption maximum at 540 nm; for other quenchers, the triplets were monitored at 480 nm. The higher ratio of molar absorption coefficients of triplet vs ketyl radical absorption at 480 nm, compared to the corresponding ratio at 540 nm, made 480 nm a more favorable monitoring wavelength than the maximum of the triplet at 540 nm.

The pseudo-first order rate constants, k_{obs}, were calculated using eq 2 taking into account concomitant, underlying growing in of the photoproduct absorption

$$\ln\left(\frac{A - A^{\infty}}{A^0 - A^{\infty}}\right) = -k_{obs}t \qquad (2)$$

where A^0, A, and A^{∞} are the absorbance changes at times 0, t, and infinity, respectively.

As it will be shown and discussed later (Scheme II), the initial products of CB triplet quenching are the ketyl radical, K, and the ketyl radical anion, $CB^{\bullet-}$. The latter becomes protonated on a very short time scale, hundreds of nanoseconds (see for example Figure 5A). Fortunately, in the spectral region of 480 nm the values of molar absorption coefficients for K and $CB^{\bullet-}$ are very similar (the isosbestic point for K/$CB^{\bullet-}$ pairs appears at about 490 nm).[30] Therefore, during monitoring of triplet decays, no significant corrections were necessary for the fast protonation of ketyl radical anions.

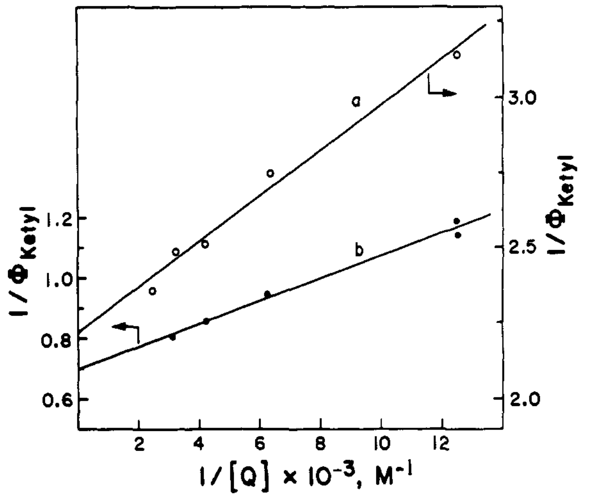

Figure 2. Plots of $1/\Phi_{ketyl}$ vs the reciprocal of concentration of (a) α-methylmethionine and (b) thiaproline (according to eq 12) in aqueous solution at pH = 6.8.

Some typical plots based on eq 1 are presented in Figure 1, and the quenching rate constants obtained for all the sulfur-containing amino acids used are summarized in Table I. The k_q at pH 6.8 for alanine, an amino acid which does not contain sulfur, was <6 × 10^5 M^{-1} s^{-1}.

Yields of CB ketyl radical (overall limiting quantum yields $\Phi_{\text{ketyl}}^{\text{lim}}$) and quenching rate constants, k_q, were determined from the modified Stern–Volmer equation (eq 12, vide infra) by monitoring changes of absorption at 570 nm for various concentrations of amino acid. Taking values of molar absorption coefficients at 570 nm for the CB triplet and the ketyl radical in aqueous solution from ref 30 (ϵ_T = 4350 M^{-1} cm^{-1} and ϵ_K = 5500 M^{-1} cm^{-1}) and using the following equation

$$\Phi_{\text{ketyl}} = \frac{\Delta A_K^{\infty}\epsilon_T}{\Delta A_T^0 \epsilon_K} \qquad (3)$$

allowed determination of the values of ketyl radical yield for the particular concentration of amino acid. In eq 3, ΔA_T^0 is an absorption change immediately after the flash, corresponding to the initial formation of triplet, and ΔA_K^{∞} is an absorption change after reaching a plateau value, corresponding to the total formation of ketyl. Assuming[30] that the quantum yield of CB triplet formation is equal to 1.0, the $\Phi_{\text{ketyl}}^{\text{lim}}$ values represent the limiting overall quantum yields of ketyl formation. As in the case of triplet decays, the corrections for the fast protonation of ketyl radical anions are not necessary since the values of molar absorption coefficients are very similar for K and $CB^{\bullet-}$ at 570 nm.[30]

Typical plots according to the modified Stern–Volmer equation (eq 12) are presented in Figure 2, and data obtained for all amino acids used are summarized in Table I.

2. Transient Absorption Spectra of Intermediates. CB Triplet, Ketyl Radical, Ketyl Radical Anion, and Intermolecular S∴S Bonded Radical Cation. The spectra of the CB triplet and the ketyl radical were obtained by flash excitation at 337 nm of CB (2 × 10^{-3} M) in the presence of various amino acids (about 1 × 10^{-3} M) in aqueous solution at pH = 6.8 and were found to be identical with those previously published by Inbar at al.,[30] λ_{max} = 540 nm for the triplet and λ_{max} = 570 nm for the ketyl radical.

Two additional intermediates could be identified from a composite spectrum (Figure 3a) taken when higher concentrations of amino acid (2 × 10^{-2} M) were used to rapidly quench more than 99% of the triplets. The composite spectrum is reminiscent of the ketyl radical spectrum (Figure 3b), but it has shoulders that makes it broader than the spectrum of the ketyl radical. The short-wavelength shoulder is in the region of 480 nm, the absorption maximum of the intermolecular S∴S bonded radical

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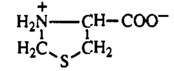
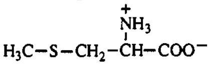
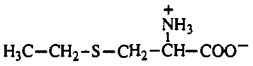
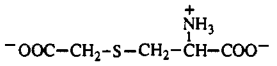
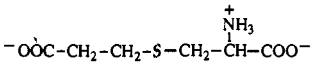
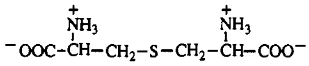
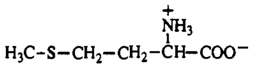
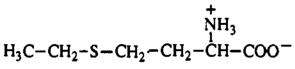
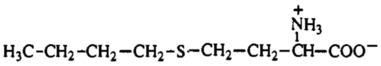
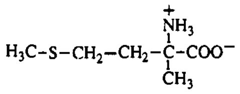
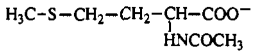
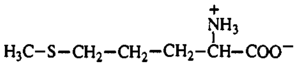
Table I. Quenching of 4-Carboxybenzophenone Triplet by Amino Acids in Aqueous Solution at pH = 6.8 at Room Temperature

			$k_q \times 10^{-9}$ (M^{-1} s^{-1})		
no.	amino acid	formula	from eq 1[a]	from eq 12[b]	Φ_{ketyl}^{lim} [c]
1	thiaproline		2.1	1.8	1.40
2	*S*-methylcysteine	 $H_3C-S-CH_2-\overset{\overset{+}{N}H_3}{CH}-COO^-$	1.8	1.4	0.59
3	*S*-ethylcysteine	 $H_3C-CH_2-S-CH_2-\overset{\overset{+}{N}H_3}{CH}-COO^-$	2.1	≈1.0	0.60
4	*S*-(carboxymethyl)-cysteine	 $^-OOC-CH_2-S-CH_2-\overset{\overset{+}{N}H_3}{CH}-COO^-$	0.81	1.2	0.82
5	*S*-(2-carboxyethyl)-cysteine	 $^-OOC-CH_2-CH_2-S-CH_2-\overset{\overset{+}{N}H_3}{CH}-COO^-$	0.97	1.3	0.74
6	lanthionine	 $^-OOC-\overset{\overset{+}{N}H_3}{CH}-CH_2-S-CH_2-\overset{\overset{+}{N}H_3}{CH}-COO^-$	0.18	≈0.4	0.48
7	methionine	 $H_3C-S-CH_2-CH_2-\overset{\overset{+}{N}H_3}{CH}-COO^-$	2.6	1.5	0.58
8	ethionine	 $H_3C-CH_2-S-CH_2-CH_2-\overset{\overset{+}{N}H_3}{CH}-COO^-$	2.9	2.6	0.52
9	buthionine	 $H_3C-CH_2-CH_2-CH_2-S-CH_2-CH_2-\overset{\overset{+}{N}H_3}{CH}-COO^-$	2.7	2.5	0.50
10	α-methylmethionine	 $H_3C-S-CH_2-CH_2-\underset{CH_3}{\overset{\overset{+}{N}H_3}{C}}-COO^-$	2.6	2.8	0.45
11	*N*-acetylmethionine	 $H_3C-S-CH_2-CH_2-\underset{HNCOCH_3}{CH}-COO^-$	1.6	2.2	0.59
12	homomethionine	 $H_3C-S-CH_2-CH_2-CH_2-\overset{\overset{+}{N}H_3}{CH}-COO^-$	2.9	3.6	0.42

[a] Estimated errors (taken as twice the standard deviations from the least-squares fits) ~5% for all amino acids except **6** (10%). [b] Estimated errors ≤30% (calculated based on twice the standard deviations of the slope and intercept). [c] Limiting quantum yield calculated from the intercept of eq 12; estimated errors ≤10% (calculated based on twice the standard deviation of intercept).

cation; see Figure 3d. The long-wavelength shoulder is in the region of 660 nm, the absorption maximum of the ketyl radical anion $CB^{\bullet -}$; see Figure 3e.

The analysis of the composite spectrum can be made quantitative in the following manner. Molar absorption coefficients (ϵ) of the ketyl radical and the radical anion have been given over a wide wavelength range by Inbar et al.[30] Analogous data are available for the S∴S-bonded radical cation of methionine obtained in the pulse radiolysis.[33] With ϵ's for all three species at three different wavelengths (λ), three equations of the form $\Delta A(\lambda) = \Sigma\epsilon_i(\lambda)c_i l$ can be solved for the three unknown concentrations (c_i). The subscript i runs from 1 to 3, l is the optical path length, and $\Delta A(\lambda)$ is taken from the composite spectrum at wavelengths corresponding to the known ϵ's. Once the concentrations of the three species were known, the three transient spectra (curves 3b, 3d, and 3e) were normalized to the ΔA corresponding to their absorption in the composite spectrum.

Spectra 3a and 3b were subtracted to remove the contribution of the ketyl radical to the composite spectrum. Before the subtraction, spectrum 3b, from ref 30, was extended toward the red by appending the renormalized tail of spectrum 3f. The resulting spectrum 3c shows the remnants of both the radical anion near 660 nm and the $(S∴S)^+$ dimer near 480 nm. In an additional analysis, spectra 3d and 3e were added together over the limited spectral range where both absorptions are well-known. This spectral sum gave a spectrum that was the same, within experimental error, as spectrum 3c. There was a deviation in the short-wavelength region which is also evident in the difference between spectra 3c and 3d in Figure 3. This discrepancy in the 400 nm region can be explained by the absorption of products other than the $(S∴S)^+$ dimer formed in the pulse radiolysis of methionine.[33,34] The quantitative agreement in this spectral analysis supports the contention that both $(S∴S)^+$ and $CB^{\bullet -}$ are products of the quenching process.

The formation of $(S∴S)^+$ dimers, similar to the case of methionine presented in Figure 3, was also found for the CB-sensitized photooxidation of amino acids **8**–**12** with relatively long-lived sulfur-centered radical cations. A fast decarboxylation and/or deprotonation of $S^{\bullet +}$ radical cation can be responsible for the lack of detection of $(S∴S)^+$ dimers for amino acids **1**–**6** (see Scheme II). Curve 3f in Figure 3 shows that at 0.5 μs almost all of the radical anion has decayed but that there is still some $(S∴S)^+$ dimer present.

3. Ketyl Radical Anion Protonation Kinetics. When triplet lifetimes could be shortened to less than 100 ns (Figure 4A′) by using high concentrations of amino acids (2×10^{-2} M), the growth and decay of $CB^{\bullet -}$ anions could be monitored in the long-wavelength region of 600–720 nm. This is illustrated in Figure 4B′, where the results of the laser flash photolysis of CB in the presence of *S*-(carboxymethyl)cysteine (2×10^{-2} M) in aqueous solution at pH = 6.1 without buffer were shown as a typical example. Since the $(S∴S)^+$ dimer is not observed for *S*-(carboxymethyl)-

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[33] Hiller, K.-O.; Masloch, B.; Göbl, M.; Asmus, K.-D. *J. Am. Chem. Soc.* **1981**, *103*, 2734–2743.

[34] Mönig, J.; Göbl, M.; Asmus, K.-D. *J. Chem. Soc., Perkin Trans. 2* **1985**, 647–651.

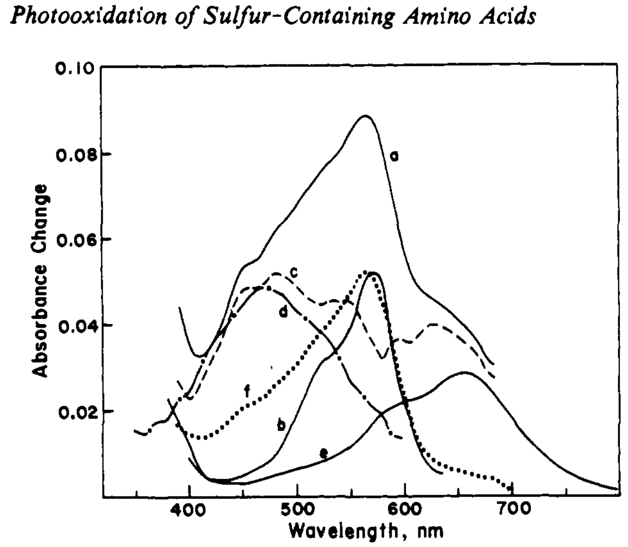

Figure 3. Transient absorption spectra of the intermediates following the quenching of 4-carboxybenzophenone triplet by methionine (20 mM) in aqueous solution at pH = 6.8: (a) immediately following the triplet decay, (b) ketyl radical spectrum taken from ref 30 (see text for computation of ΔA_{570}), (c) difference of spectrum a and b, with b extended at long wavelength (see text), (d) spectrum of $(>S\therefore S<)^+$ obtained from the pulse radiolysis of methionine in aqueous solution at pH = 1.0 (see text for computation of ΔA_{480}), (e) ketyl radical anion spectrum taken from ref 30 (see text for computation of ΔA_{660}), (f) taken 0.5 μs after the flash.

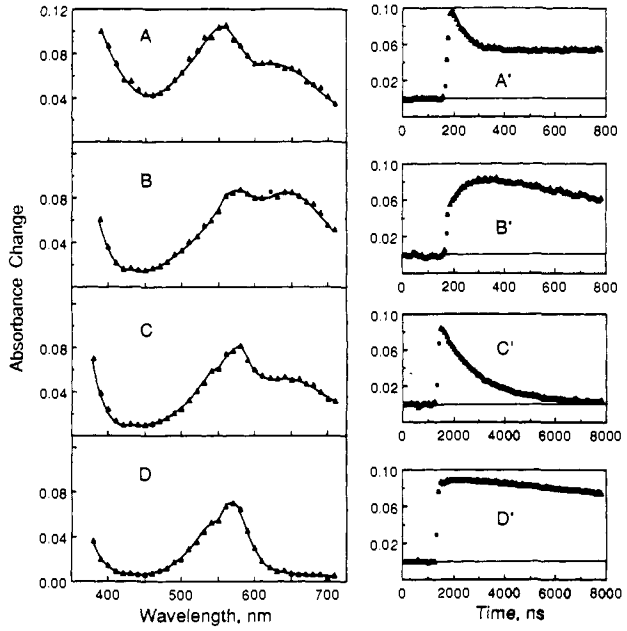

Figure 4. Transient absorption spectra and kinetic traces following laser flash photolysis of CB (2 mM) in the presence of *S*-(carboxymethyl)cysteine (20 mM) in aqueous solution at pH = 6.1 (no buffer): (A) 8 ns, (B) 120 ns, (C) 1.0 μs, (D) 6 μs, (A') λ = 540 nm, (B' and C') λ = 660 nm, (D') λ = 570 nm.

cysteine, the spectra presented in Figure 4 can be inferred to consist of the CB triplet, the ketyl radical, and the ketyl radical anion, depending on delay times after the flash. As shown in Figure 4A, the transient spectrum at the initial stage of the growth process (several nanoseconds after the flash) was likely due to the ketyl radical (λ_{max} = 570 nm), along with the contribution from the CB triplet (λ_{max} = 540 nm) and the CB$^{\bullet-}$ anion radical (λ_{max} = 660 nm). Both K and CB$^{\bullet-}$ intermediates are shown to be primary photoproducts of the quenching of the CB triplet by amino acids containing a sulfur atom. Kinetic traces C' and D' (Figure 4) enable us to calculate concentrations of K and CB$^{\bullet-}$ formed in the primary photochemical process, and thus to estimate the contributions of the proton-transfer process within the CT complex (35%) and the diffusion-apart process (65%) (Scheme II). After the decay of CB$^{\bullet-}$ (Figure 4C'), the ketyl radical is the only species observed (Figure 4D). A comparison of kinetic traces monitored at 660 nm (Figure 4C'), where CB$^{\bullet-}$ is the only absorbing species, and at 570 nm (Figure 4D'), where both K and CB$^{\bullet-}$ absorb with similar molar absorption coefficients,[30] clearly indicates that the decay of CB$^{\bullet-}$ leads to the formation of ketyl radicals that are additional to those produced in the reaction within the CT complex (Scheme II, vide infra).

The decay kinetics of the ketyl radical anion CB$^{\bullet-}$ monitored at λ_{max} = 660 nm strongly depend on experimental conditions used, e.g. pH, presence of phosphate buffer, and concentration of amino acid. All these data, together with the weak acidity ($pK_a = 8.2$)[30] of the CB ketyl radical, strongly suggest that the protonation of the CB$^{\bullet-}$ anion, produced as a result of the CB triplet quenching by amino acids, is observed (Scheme II).

The influence of the buffer, the concentration of the amino acid, and the pH on the decay kinetics of the CB$^{\bullet-}$ anion is presented in Figure 5. The presence of phosphate buffer (0.025 M) increases the value of the rate constant of CB$^{\bullet-}$ decay about 14 times at constant pH (Figures 5, parts A and B). A comparison of kinetic traces obtained from the pulse radiolysis of CB (Figure 5D) with that from the flash photolysis of CB at high concentrations of amino acid (Figure 5B) clearly indicates an additional reaction of methionine (vide infra) with the CB$^{\bullet-}$ anion to form ketyl radicals.

The rate constant for protonation of ketyl radical anion was determined by monitoring decays of the CB$^{\bullet-}$ anion at 660 nm obtained from the flash photolysis of CB (2×10^{-3} M) and methionine (2×10^{-2} M) in unbuffered aqueous solution varying in pH between 7.0 and 5.2. The appropriate values of pH were adjusted by adding NaOH and/or $HClO_4$. Typical examples of the influence of the pH of the solution on the decay kinetics of CB$^{\bullet-}$ are presented in Figure 5, parts B and C. A plot of the observed rate constants for CB$^{\bullet-}$ decays vs concentration of H^+ allowed us to determine the second-order rate constant of protonation of the ketyl radical anion in aqueous solution at room temperature, $k = (5 \pm 1) \times 10^{10}\ M^{-1}\ s^{-1}$. Experimental limitations, i.e. the protonation reaction of the CB$^{\bullet-}$ anion by methionine at high concentration and a precipitation of CB in acidic solution (pH < 5.2), restricted the accuracy of the measurements of the protonation rate constant. However, the value obtained is in reasonable agreement with that measured in the pulse radiolysis studies of CB in aqueous solution $k = (6.8 \pm 0.1) \times 10^{10}\ M^{-1}\ s^{-1}$.[35]

4. Secondary Reaction of the CB Ground State. When the concentration of amino acids is high enough such that the CB triplet decay is virtually complete in less than 100 ns, the experimental kinetic traces clearly indicate two processes of ketyl radical formation. A fast decay component occurs on a nanosecond time scale within the time scale of the triplet decay. This fast decay can be attributed to two independent channels, both of which start with an electron transfer from the sulfur atom to the CB triplet within the CT complex (Scheme II). The first channel involves a fast protonation of the ketyl radical anion within the complex. The second channel involves diffusion apart of the ketyl radical anion and the radical cation followed by a fast protonation of the ketyl radical anion by hydrogen ions, ground-state amino acids, or $H_2PO_4^-$. A slow process occurs on the microsecond time scale, and its rate constant depends on the concentration of CB in the ground state.

Typical experimental traces observed for monitoring the formation of ketyl radical (λ_{max} = 570 nm) are presented in Figure 6A. After the formation of ketyl radical in the fast process, a slow process of ketyl radical formation is observed when thiaproline is the quencher (curve a of Figure 6A). This slow growth does not appear when *S*-(carboxymethyl)cysteine (vide infra) is the amino acid present (curve b of Figure 6A). As will be discussed later, the slow process of ketyl radical formation can be assigned to the one-electron reduction of CB in the ground state by the α-aminoalkyl radicals produced from the decarboxylation of the sulfur-centered radical cation (Scheme II). To support this

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(35) Bobrowski, K.; Marciniak, B. *Radiat. Phys. Chem.*, to be submitted.

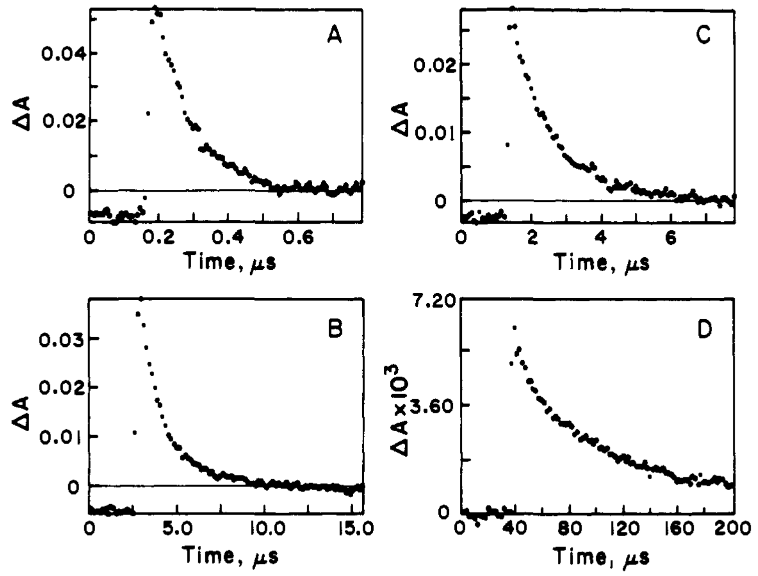

Figure 5. Kinetic traces following the laser flash photolysis for decay of $CB^{\bullet -}$ ($\lambda = 660$ nm) in aqueous solution containing CB (2 mM) and methionine (20 mM): (A) pH = 6.8 with phosphate buffer [NaH_2PO_4 (0.025 M) and Na_2HPO_4 (0.025 M)], (B) pH = 6.7 without buffer, (C) pH = 5.2 without buffer, (D) kinetic trace following pulse radiolysis of CB (2 mM) in aqueous solution (N_2 saturated) at pH = 7.3 (no methionine).

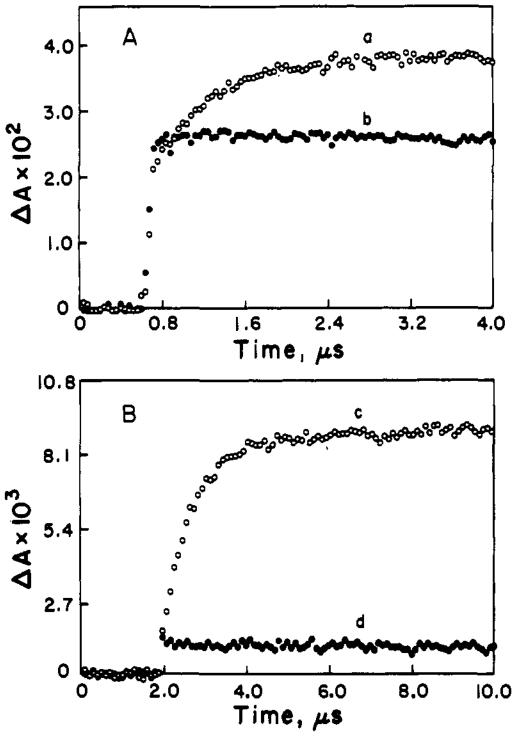

Figure 6. Kinetic traces for the slow growth of ketyl radical formation at 570 nm observed in (A) flash photolysis in aqueous solution at pH = 6.8, (a) thiaproline (20 mM, [CB] = 2 mM), (b) S-(carboxymethyl)-cysteine (20 mM, [CB] = 2 mM), and (B) pulse radiolysis in N_2O-saturated aqueous solution at pH = 6.0, (c) thiaproline (20 mM, [CB] = 2 mM), (d) 2,2'-thiodiethanoic acid (20 mM, [CB] = 2 mM).

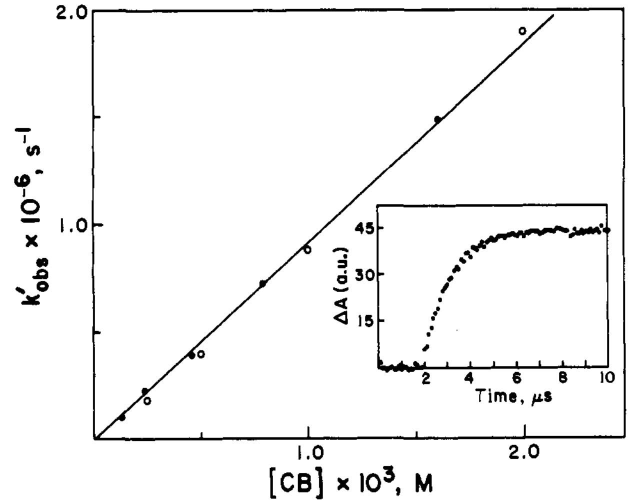

Figure 7. 4-Carboxybenzophenone (CB) concentration dependence of the pseudo-first-order rate constant, k'_{obs}, for the reaction of CB ground state with α-aminoalkyl radical derived from thiaproline. Points represent results from a pulse radiolysis (N_2O-saturated aqueous solution at pH = 5.8, [thiaproline] = 20 mM); circles represent results from a flash photolysis (aqueous solution pH = 6.8, [thiaproline] = 20 mM). Insert: a kinetic trace for the growth of ketyl radical formation observed at 570 nm in pulse radiolysis for [CB] = 1.6 mM.

mechanism complementary pulse radiolysis experiments were performed in which the appropriate α-aminoalkyl-type radicals were generated, and their reaction with the CB ground state was monitored. The formation of ketyl radicals was observed, as in the case of flash photolysis, by monitoring of the absorption of ketyl radical at 570 nm. The examples of such studies are presented in Figure 6B. The lack of a slow process of ketyl radical formation for 2,2'-thiodiethanoic acid (curve d of Figure 6B), where decarboxylation leads to α-(alkylthio)alkyl radicals[36] but not to α-aminoalkyl radicals, is in line with the proposed mechanism for the secondary process of ketyl radical formation.

The slow process of ketyl radical formation can be characterized by pseudo-first-order rate constants, k'_{obs}, that are linearly dependent on the CB ground-state concentration. Figure 7 shows a representative plot obtained from both flash photolysis and pulse radiolysis experiments for thiaproline (2×10^{-2} M) and various concentrations of CB (2×10^{-4}–2×10^{-3} M). The slope of this plot gives the bimolecular rate constant, k_{10}, for the one-electron reduction of 4-carboxybenzophenone by α-aminoalkyl radicals produced from thiaproline. Using a similar procedure, k_{10} values for other amino acids were determined and summarized in Table II. As the data in Table II show, the values of k_{10} do not depend significantly on the structure of the α-aminoalkyl-type radical. However, there is a small effect of electron-withdrawing group on the magnitude of k_{10}, as in the case of N-acetylmethionine (**11**).

The absorption spectra of α-aminoalkyl radicals (αN) for two representative amino acids, thiaproline and S-methylcysteine, and their kinetic traces from pulse radiolysis are presented in Figure 8. The absorption of the αN radicals appears in the short-

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[36] Bobrowski, K.; Schöneich, Ch.; Asmus, K.-D. Unpublished results.

Table II. Quantum Yield of Ketyl Radical Formation in the Photochemical Processes and Kinetic Parameters for the Secondary Reactions of α-Aminoalkyl Radicals

amino acid	$k_{10} \times 10^{-9}$ [a] ($M^{-1}\,s^{-1}$)	η [b]	Φ'_{ketyl} [c]	Φ_α [d]	Φ''_{ketyl} [e]
1	0.93	1.00	0.80	0.60	0.60
2	1.27	0.76	0.48	0.15	0.11
3	1.08	0.64	0.51	0.15	0.09
4	0.78	0.94	(0.76, 0.82)	≤0.07	≤0.06
5	0.96	0.78	0.64	0.13	0.10
6	*f*				
7	0.80	0.94	0.33	0.27	0.25
8	0.73	0.90	0.33	0.21	0.19
9	*f*				
10	1.04	1.00	≈0.32	≈0.13	≈0.13
11	0.56	0.27	(0.53, 0.59)	≤0.20	≤0.06
12	1.19	1.00	(0.35, 0.42)	≤0.07	≤0.07

[a] Rate constant for reaction of α-aminoalkyl radicals with the 4-carboxybenzophenone ground state obtained from the pulse radiolysis studies, estimated error ≤20%. [b] Fraction of α-aminoalkyl radicals forming ketyl radicals in the reaction with 4-carboxybenzophenone ground state for [CB] = 2×10^{-3} M (eq 15). [c] Efficiency of ketyl radical formation in the photochemical process for [Q] = 2.0×10^{-2} M (eq 13), estimated error ≤30%. [d] Efficiency of α-aminoalkyl radicals formation (eq 16), estimated error ≤30%. [e] Efficiency of ketyl radical formation in the secondary reactions equal to $\eta\Phi_a$. [f] Solubility of amino acid too low.

wavelength region ($\lambda_{max} < 300$ nm). A similar spectrum was observed for methionine.[24] Observations in this spectral region could not be made in the flash photolysis studies because of the CB ground-state absorption below 370 nm. As shown in insets of Figure 8, lifetimes of α-aminoalkyl radicals depend significantly on the structure of the radicals. Since the formation of ketyl radicals via the reaction (k_{10}) of α-aminoalkyl radicals is in competition with the decay (k_{11}) of the α-aminoalkyl radicals, the efficiency of formation of ketyl radicals via the dark mechanism can be expressed as $\eta = k_{10}[CB]/(k_{10}[CB] + k_{11})$ (see Discussion). The η values obtained in the pulse radiolysis for all amino acids used are summarized in Table II. With the exceptions of amino acids **2**, **3**, **5**, and **11**, the η values are close to unity when [CB] = 2×10^{-3} M.

The overall quantum yield of ketyl radical formation Φ_{ketyl} consists of two terms: the quantum yield of ketyl radical formation in the photochemical process (from the fast component), Φ'_{ketyl}, and the yield of ketyl radical formation in the secondary reactions (from slow growth), Φ''_{ketyl}. Φ''_{ketyl} can be determined from the kinetic traces for the slow growth of ketyl radicals for relatively high concentrations of amino acid (2×10^{-2} M) and CB (2×10^{-3} M) by taking the appropriate values of Φ^{lim}_{ketyl} from Table I as an actinometer. For amino acids **7**–**12**, to minimize absorption by the (S∴S)$^+$ dimers, the kinetic traces were monitored at 370 nm, instead of 570 nm used for amino acids **1**–**6**. All these data, including calculated quantum yields of α-aminoalkyl radical formation, Φ_α, are summarized in Table II.

Discussion

Based on the experimental results, we propose a reaction mechanism (Scheme II) for the 4-carboxybenzophenone-sensitized photooxidation of sulfur-containing amino acids in aqueous neutral solution. The initial step in this mechanism is an electron transfer from a sulfur atom to the CB triplet to form a [CB$^{\bullet-}$···Q$^{\bullet+}$] complex. The complex breaks up through three competing pathways: (1) proton transfer within the complex (k_H) and diffusion apart of the radicals, (2) diffusion apart ($k_{diff\,apart}$) of the ions, and (3) back electron transfer (k_{bET}). The first reaction leads to the formation of ketyl radicals and α-(alkylthio)alkyl radicals, the second leads to the formation of separated radical anions CB$^{\bullet-}$ and sulfur-centered radical cations, and the third leads back to the starting materials, i.e. CB ground state and amino acid.

The evidence for the separation of the complex directly into ions is supported by the observation of the CB$^{\bullet-}$ anion in the transient absorption spectra at λ = 660 nm, see Figure 3. Since the pK_a of the CB ketyl radical is 8.2,[30] the CB$^{\bullet-}$ is ultimately protonated in aqueous solution (pH = 6.8) to produce neutral ketyl radicals. The protonation was quite rapid under most experimental conditions used in this work because CB$^{\bullet-}$ could be protonated by the phosphate buffer and/or amino acids (see Figure 5) in addition to hydrogen ions.

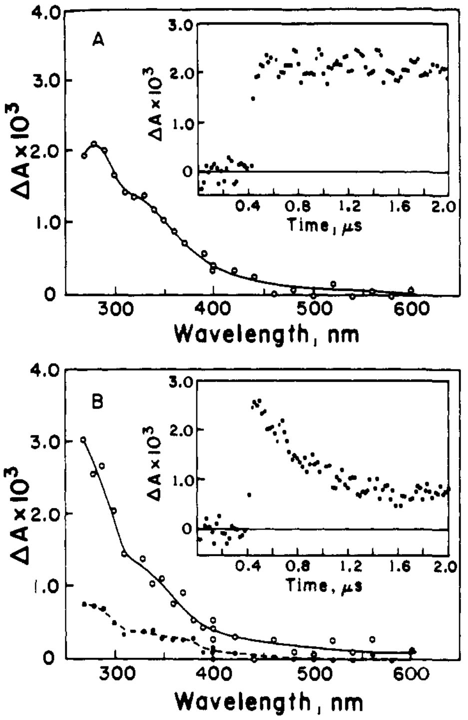

Figure 8. Absorption spectra of α-aminoalkyl radicals derived from (A) thiaproline (1.4 μs after the pulse) and (B) *S*-methylcysteine (upper curve 70 ns after the pulse; lower curve 1.4 μs after the pulse) obtained in the pulse radiolysis of N_2O-saturated aqueous solution at pH = 5.3. Inserts: kinetic traces recorded at 280 nm.

The presence of S$^{\bullet+}$ radical cation was established by the detection of (S∴S)$^+$ dimer formed at high concentrations of amino acids **7**–**12**; see Figure 3. In addition to the reaction with amino acid [(S∴S)$^+$ formation], the S$^{\bullet+}$ radical cation can also undergo decarboxylation to form the α-aminoalkyl radical. The latter reaction is the main pathway for amino acids **1**–**6** ($H_3N^+CH(COO^-)(CH_2)_nSR$), where the structure of the amino acids (n = 1) seems to favor fast intramolecular electron transfer (Scheme II). Indirect evidence that this process was occurring was the observation of the slow growth of ketyl radical absorption (see Figure 6) and the lack of the observation of (S∴S)$^+$ dimer absorption. Complementary pulse radiolysis experiments showed that the only likely source of the excess ketyl radicals could be the reaction of αN radicals with ground-state CB; see Figure 6 and accompanying text.

As a quantitative framework for the discussion, it is helpful to list the pertinent reactions 4–11 (derived from Scheme II) to account for the formation of the ketyl radical in aqueous solution at pH = 6.8 (buffer). Fast protonation of CB$^{\bullet-}$ anion radicals under the experimental conditions used (vide supra) and similar values of molar absorption coefficients for K and CB$^{\bullet-}$ at the monitoring wavelengths (vide supra) allowed us to simplify the kinetic scheme to reactions 4–11.[37]

On the basis of this scheme, using the steady-state approximation, the following expression for Φ_{ketyl}^{-1} as a function of $[Q]^{-1}$

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(37) Reaction 9 is equivalent to the back electron transfer, reaction k_{bET} in Scheme II; reaction 7 represents only that part of the diffusion-apart reaction leading to the formation of αN radicals, whereas reaction 8 represents both the proton-transfer reaction within the [CB$^{\bullet-}$···Q$^{\bullet+}$] complex (k_H) and that part of reaction $k_{diff\,apart}$ which is not included in reaction 7. It is important to note that the sum of $k_H + k_{diff\,apart}$ (Scheme II) is equal to the sum of $k_7 + k_8$ (since there is fast protonation of CB$^{\bullet-}$ under the experimental conditions used).

$$\mathrm{CB} \xrightarrow{h\nu} {}^1\mathrm{CB}^* \xrightarrow{\Phi_{\mathrm{ISC}} = 1} {}^3\mathrm{CB}^* \tag{4}$$

$$ {}^3\mathrm{CB}^* \xrightarrow{k_2} \mathrm{CB} \tag{5}$$

$$ {}^3\mathrm{CB}^* + \mathrm{Q} \xrightarrow{k_q} [\mathrm{CB}^{\bullet-} \cdots \mathrm{Q}^{\bullet+}] \tag{6}$$

$$[\mathrm{CB}^{\bullet-} \cdots \mathrm{Q}^{\bullet+}] \xrightarrow{k_7} \mathrm{K} + \alpha\mathrm{N} + \mathrm{CO_2} \tag{7}$$

$$[\mathrm{CB}^{\bullet-} \cdots \mathrm{Q}^{\bullet+}] \xrightarrow{k_8} \mathrm{K} + \text{Products} \tag{8}$$

$$[\mathrm{CB}^{\bullet-} \cdots \mathrm{Q}^{\bullet+}] \xrightarrow{k_9} \mathrm{CB} + \mathrm{Q} \tag{9}$$

$$\alpha\mathrm{N} + \mathrm{CB} \xrightarrow{k_{10}} \mathrm{K} + \text{Products} \tag{10}$$

$$\alpha\mathrm{N} \xrightarrow{k_{11}} \text{Products} \tag{11}$$

(the modified Stern-Volmer relation) can be derived:

$$\frac{1}{\Phi_{\mathrm{ketyl}}} = \frac{1}{\Phi'_{\mathrm{ketyl}} + \Phi''_{\mathrm{ketyl}}}\left[1 + \frac{1}{k_q\tau_T[\mathrm{Q}]}\right] \tag{12}$$

where Φ'_{ketyl} is the efficiency of ketyl radical formation in the photochemical processes

$$\Phi'_{\mathrm{ketyl}} = \frac{k_7 + k_8}{k_7 + k_8 + k_9} \tag{13}$$

Φ''_{ketyl} is the efficiency of ketyl radical formation in the secondary reactions

$$\Phi''_{\mathrm{ketyl}} = \eta\Phi_\alpha \tag{14}$$

η is the fraction of αN radicals forming ketyl radicals in the reaction with 4-carboxybenzophenone in the ground state

$$\eta = \frac{k_{10}[\mathrm{CB}]}{k_{10}[\mathrm{CB}] + k_{11}} \tag{15}$$

and Φ_α is the efficiency of αN radical formation

$$\Phi_\alpha = \frac{k_7}{k_7 + k_8 + k_9} \tag{16}$$

At relatively high concentrations of quencher, when more than 95% of the triplets are quenched, the Φ'_{ketyl} and Φ''_{ketyl} values can be treated as quantum yields.

The kinetic data in Table I for various sulfur-containing amino acids show that these compounds are relatively good quenchers of the CB triplet. The values of k_q are in the range of 1.8×10^8–2.9×10^9 M^{-1} s^{-1} and vary with the structure of the amino acids used. The steady-state experiments performed by Cohen and Ojanpera[8] for **7** and **11** at pH = 7 led to the values of k_q in the range of our results (1.2×10^9 M^{-1} s^{-1} and 1.5×10^9 M^{-1} s^{-1}, respectively). The k_q values obtained previously for the quenching of the benzophenone triplet by methionine by other authors are also in good agreement with our result ($k_q = 2.4 \times 10^9$ M^{-1} s^{-1} for 4:1 water/acetonitrile[5] and $k_q = 3.5 \times 10^9$ M^{-1} s^{-1} for aqueous solution[6] at pH = 7).

A comparison of k_q values in the homologous series of amino acids, i.e. **2**, **7**, and **12**, shows the increase of the k_q values with the distance between the sulfur atom and the electron-withdrawing $\mathrm{CH(NH_3^+)COO^-}$ group. This type of behavior is in agreement with the electron-transfer mechanism of quenching (from the sulfur atom to the CB triplet). In this and the following structure/k_q correlations, the more precisely known k_q's in column 4, not column 5, of Table I could be consulted.

A comparison of the k_q values (compensated for the effect of reactant charge)[38] for **5**, **6**, and 3,3′-thiodipropionic acid (1.0×10^9 M^{-1} s^{-1}) suggests that the introduction of electron-withdrawing groups, $\mathrm{NH_3^+}$, on the carbons β to the sulfur leads to a decrease of the quenching rate constant, as is expected for electron-transfer quenching. However, the introduction of the second $\mathrm{NH_3^+}$ group (for **6**) leads to a larger relative decrease of k_q than the first, which may suggest the contribution of a steric factor.

The effect of alkyl groups attached to the sulfur atom on the quenching rate constants can be illustrated by a comparison of k_q values for **2** and **3**, or **7** and **8**. These data are also in agreement with the proposed mechanism of quenching, i.e. electron transfer from the sulfur atom to the CB triplet, which should be enhanced by the electron-donating character of the alkyl groups.

The small value of $k_q < 6 \times 10^5$ M^{-1} s^{-1} for the zwitterionic form of alanine indicates that the COO$^-$ moiety is not responsible for the efficient reduction of the carbonyl triplets in these experiments. A similar value of $k_q < 5 \times 10^5$ M^{-1} s^{-1} was reported[5] for quenching of benzophenone triplets by glycine, another non-sulfur-containing amino acid, at pH 7.0 in 4:1 water/acetonitrile.

As shown in Table I, the values of k_q obtained from the monitoring of the ketyl quantum yield Φ_{ketyl} as a function of quencher concentration (the modified Stern–Volmer equation, eq 12) are equal within experimental error to those obtained from the direct triplet quenching measurements (eq 1). This is an additional justification for the validity of the assumptions underlying the kinetic scheme (eqs 4–11).

The values of $\Phi^{\mathrm{lim}}_{\mathrm{ketyl}}$ in Table I, representing the limiting overall quantum yield of ketyl radical formation in both the photochemical and the dark reactions, are in the range of 0.4–1.4, depending on the amino acid used. They are higher than those obtained for aliphatic thioethers.[25,26] A comparison of the $\Phi^{\mathrm{lim}}_{\mathrm{ketyl}}$ value obtained for methionine (Table I) with those determined previously[5,6,8] shows excellent agreement.

In the case of thiaproline, the $\Phi^{\mathrm{lim}}_{\mathrm{ketyl}}$ value is equal to 1.4 (the only $\Phi^{\mathrm{lim}}_{\mathrm{ketyl}}$ higher than 1.0 for the amino acids used). It consists of two terms: Φ'_{ketyl} (eq 13) and Φ''_{ketyl} (eq 14), determined to be 0.80 and 0.60, respectively (Table II). Calculations of $\mathrm{CB^{\bullet-}}$ and K concentrations formed in the primary photochemical process, similar to those performed for **4** (vide supra), led us to estimations of the contributions from proton-transfer reactions within the CT complex (~35%) and from diffusion apart reactions (~65%) (Scheme II) that were similar to those of **4**. This clearly indicates that the contribution of the diffusion apart reaction ($k_{\mathrm{diff\,apart}}$) is the main photochemical pathway. Two supplementary measurements further support this conclusion: the value of the quantum yield of CO$_2$ formation was determined in steady-state experiments for thiaproline to be $\Phi_{\mathrm{CO_2}} = 0.53 \pm 0.13$,[39] and the value of the quantum yield of $\mathrm{CB^{\bullet-}}$ anion formation was estimated from the flash photolysis studies to be $\Phi_{\mathrm{CB^{\bullet-}}} \sim 0.55$. The latter value was estimated by monitoring the absorption changes at 660 nm, assuming that the $\mathrm{CB^{\bullet-}}$ anion is the only absorbing species at this wavelength. In this calculation, the formation of K, monitored at 570 nm, was used as the actinometer. A similar treatment of methionine gives $\Phi_{\mathrm{CO_2}} = 0.20 \pm 0.05$[39] and $\Phi_{\mathrm{CB^{\bullet-}}} \sim 0.28$. These values, together with the results from Table II, suggest that the process of the diffusion apart of the ions making up the $[\mathrm{CB^{\bullet-}}\cdots\mathrm{Q^{\bullet+}}]$ complex (Scheme II, $k_{\mathrm{diff\,apart}}$) is also dominating over the process of proton transfer within the $[\mathrm{CB^{\bullet-}}\cdots\mathrm{Q^{\bullet+}}]$ complex (k_{H}) in the case of methionine.

The values of the quantum yield of ketyl formation in the photochemical processes, Φ'_{ketyl}, are in the range of 0.3–0.8 (Table II), depending on the amino acid used. They are generally higher than those observed for aliphatic thioethers.[25,26] The competition

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[38] Steinfeld, J. I.; Francisco, J. S.; Hase, W. L. *Chemical Kinetics and Dynamics*; Prentice Hall: Englewood Cliffs, NJ, 1989; p 163.

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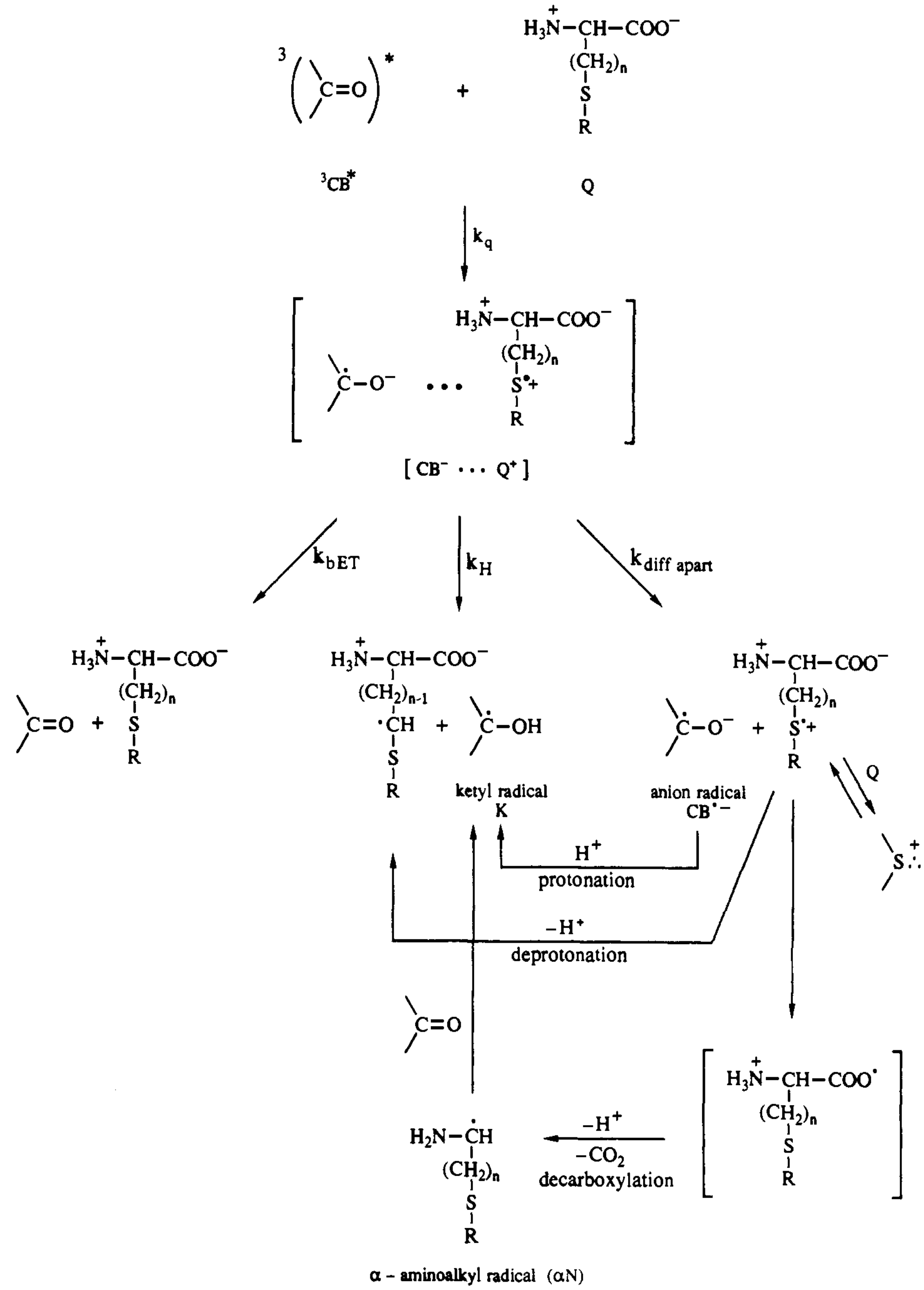
Scheme II



between photochemical pathways of ketyl radical formation (reactions 7 and 8) and a back electron transfer reaction (reaction 9) can explain the observed results.

A comparison of Φ'_{ketyl}, $\Phi_{\text{CB}^{\cdot-}}$, and Φ_α can lead to some conclusions concerning the fate of some free $S^{\cdot+}$ radical cations (Scheme II). For thiaproline **1**, diffusion apart of the ions was shown above to be the main pathway of the $[\text{CB}^{\cdot-}\cdots\text{Q}^{\cdot+}]$ decay. Since $\Phi_{\text{CB}^{\cdot-}} = \Phi''_{\text{ketyl}} = \Phi_\alpha$, the deprotonation of the free $S^{\cdot+}$ radical cations can be neglected in comparison with decarboxylation. In the other amino acids where $(S\therefore S)^+$ was not observed, **2–5**, we can not distinguish which channel(s) is (are) responsible for the low values of Φ_α. These may occur by the deprotonation of the free $S^{\cdot+}$ radical ions and/or the protonation reaction k_H. For amino acid **4**, $\Phi_{\text{CB}^{\cdot-}}$ is 0.53, which suggests that diffusion apart of the ions, and not proton transfer within the complex (k_H), is also the main pathway in this case. In a structurally related compound, 2,2′-thiodiethanoic acid, it was found in pulse radiolysis studies[36] that efficient decarboxylation of the carboxyl group located on the carbon α to the sulfur takes place. Such a reaction leads to an α-(alkylthio)alkyl radical, which can explain the low value of Φ_α for amino acid **4**, which also contains a carboxyl group on a carbon α to the sulfur (see Table II and curve b of Figure 6A).

Based on our results for the amino acids where $(S\therefore S)^+$ dimers are formed, **7–12**, it is not possible to distinguish between the k_H and $k_{\text{diff apart}}$ channels, except for **7** and **8**. In our experimental conditions ([CB] = 2×10^{-3} M, [Q] = 2×10^{-2} M), the formation of $(S\therefore S)^+$ dimers for **7–12**, and especially for **10–12**, leads to large errors in the Φ'_{ketyl} and Φ''_{ketyl} determinations. For amino acids **10–12**, the formation of ketyl radicals in the dark reaction is determined by the lifetime (τ_D) of $(S\therefore S)^+$ dimers (τ_D = 2, 50, and 10 μs, respectively, with $1/(k_{10}[\text{CB}]) < \tau_D$). This explains the low Φ''_{ketyl} yields for **10–12** (Table II). These dimer lifetimes (τ_D) are relatively long compared to the time window for the observation of ketyl radical formation in the dark reaction. The slow dissociation of the dimer shifts the equilibrium $S^{\cdot+} + Q \rightleftarrows (S\therefore S)^+$ toward the dimer. On the other hand, for amino acids **7** and **8** this equilibrium is rapidly established, and the decay of

$(S\therefore S)^+$ is determined by the decay of the $S^{\cdot+}$ radical cation, mainly by decarboxylation. The τ_D's for the dimer decay in **7** and **8** are 700 and 900 ns, respectively, and both of the τ_D's are equal to $1/(k_{10}[\mathrm{CB}])$ within experimental error (Table II). Comparison of Φ'_{ketyl} and Φ_α values for **7** and **8** shows that diffusion apart, not proton transfer, is a dominant pathway. A significant contribution of radical ions in the quenching mechanism was also found in the benzophenone-sensitized photooxidation of 1,5-dithiacyclooctane.[40]

Conclusions

4-Carboxybenzophenone (CB) sensitized photooxidation of sulfur-containing amino acids in neutral aqueous solution occurs via electron transfer from the sulfur atom to the triplet state of CB. This was established by the large values of quenching rate constants (k_q's in the range of 10^8–10^9 $\mathrm{M^{-1}\,s^{-1}}$, depending on the structure of the amino acid) and by the observation of free radical ions. In contrast to benzophenone/thioether systems,[25,26] where electron-transfer quenching is followed by intramolecular proton transfer within the CT complex (leading to the formation of α-(alkylthio)alkyl and ketyl radicals), electron-transfer quenching in CB/sulfur-containing amino acid systems is followed mainly by the diffusion apart of the CT complex (resulting in efficient formation of sulfur-centered radical cations and ketyl radical anions). The fast formation of ketyl radicals is ascribed mainly to the protonation of the initially produced ketyl radical anions (the proton transfer reaction within the CT complex, as it was shown for **1** and **4**, may lead to a contribution of about 35%). The slow formation of the ketyl radicals, which occurs on a microsecond time scale, is assigned to the one-electron reduction of the CB ground state by the α-aminoalkyl-type radicals. This was established by the linear dependence of the pseudo-first-order formation rate constants on the CB ground-state concentration, generation of the appropriate α-aminoalkyl radicals, and kinetic studies in the complementary pulse radiolysis experiments.

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Polymorphism in Anthranilic Acid: A Reexamination of the Phase Transitions

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Abstract: The three known polymorphs of anthranilic acid (2-aminobenzoic acid) have been examined by X-ray diffraction photography and infrared spectroscopy. The primary product of the phase transition occurring in heated crystals of polymorph I appears to be polymorph III, not polymorph II as commonly reported. The polycrystalline product III phase reverts to I on grinding. The III → I transition is also observed when an authentic sample of III is subjected to vigorous grinding. The occurrence of a II → I solid-state transition, the subject of disagreement in the literature, has been observed in samples of II subjected to vigorous grinding and sometimes spontaneously in single crystals of II. The product phase in the single-crystal II transition can be identified as I by its discrete X-ray reflections. The particular conditions required for initiating the II → I transition in intact single crystals of II have not yet been determined.

Introduction

As we have conducted our continuing investigation of hydrogen-bonded organic cocrystals, it has become clear to us that the solid-state behavior of one specific potential cocrystal component, anthranilic acid (2-aminobenzoic acid), should be reexamined.[2] This compound is commonly found on the shelves of most chemical laboratories and is structurally simple on the molecular level, but to a remarkable degree many of its solid-state properties remain ambiguous or obscure, even after over a century of investigations involving crystallographic and spectroscopic techniques. Reports concerning the properties of these polymorphs remain widely scattered throughout the literature, and some are strikingly contradictory. In this paper, we describe the results of our own investigation of the various anthranilic acid polymorphs, focusing primarily on the solid-state phase transitions that certain of these polymorphs undergo. Results obtained in our laboratory are in conflict in several areas with those published by previous workers over a period of many years.

Crystallographic investigations of anthranilic acid[3] date back at least to 1877, when Haushofer[4] published a description of the orthorhombic modification generally known today as polymorph I. X-ray and neutron crystal structure analyses have since established that this structure contains one neutral molecule and one zwitterion in the asymmetric unit.[5] A second polymorph, the one known today as polymorph II, was examined by Steinmetz;[6] this orthorhombic modification has since been shown by X-ray structure analyses to consist entirely of neutral molecules.[7,8] Steinmetz noted that heating I caused it to undergo an apparent

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