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Excited Triplet State of *N*-(9-Methylpurin-6-yl)pyridinium Cation as an Efficient Photosensitizer in the Oxidation of Sulfur-Containing Amino Acids. Laser Flash and Steady-State Photolysis Studies

Bronislaw Marciniak,[*,†,‡] **Gordon L. Hug,**[‡] **Jaroslaw Rozwadowski,**[†] **and Krzysztof Bobrowski**[‡,§]

Contribution from the Faculty of Chemistry, A. Mickiewicz University, 60-780 Poznan, Poland, and the Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556

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Abstract: Sulfur-containing amino acids were oxidized via photosensitization by the *N*-(9-methylpurin-6-yl)pyridinium cation (Pyr^+) in neutral aqueous solutions. The mechanism of this reaction was investigated by laser flash and steady-state photolysis. Rate constants were determined for the quenching of the pyridinium cation triplet state by five sulfur-containing amino acids. For comparison, analogous rate constants were measured for the quenchers 2,2′-thiodiethanoic acid, alanine (a non-sulfur amino acid), and ammonium acetate. The rate constants for these reactions were found to be in the range of 2.1×10^8 to 7.8×10^9 $\mathrm{M}^{-1}\ \mathrm{s}^{-1}$ for all sulfur-containing compounds but were found to be 3−4 orders of magnitude lower for ammonium acetate and alanine. Time-resolved transient absorption spectra accompanying the quenching events were assigned to the excited triplet state of the pyridinium cation, $^3\mathrm{Pyr}^+$ ($\lambda = 550$ nm), the $(\mathrm{S}\therefore\mathrm{S})^+$ radical cations of some of the amino acids ($\lambda = 480$ nm), and the *N*-(9-methylpurin-6-yl)pyridinyl radical, $\mathrm{Pyr}^\bullet$ ($\lambda = 610$ nm). The presence of the latter species was separately confirmed in complementary pulse radiolysis experiments. The formation of the $\mathrm{Pyr}^\bullet$ radical occurred for some amino acids (methionine and thiaproline) in two temporally distinct processes. A fast component, that occurred on a nanosecond time scale, was ascribed to electron transfer from the sulfur atom to the triplet state of the pyridinium cation followed by diffusion apart of the CT complex. A slower formation of $\mathrm{Pyr}^\bullet$, that occurred on a microsecond time scale, was characterized by a pseudo-first-order rate constant that depended linearly on the pyridinium cation ground-state concentration ($k = 2.9 \times 10^9$ $\mathrm{M}^{-1}\ \mathrm{s}^{-1}$). This dark reaction was assigned to the one-electron reduction of the Pyr^+ ground state by α-aminoalkyl radicals. These α-aminoalkyl radicals were formed from the radical cations of the amino acids as a result of an intramolecular electron transfer from the carboxyl group to the sulfur-centered radical cation, followed by a decarboxylation. Steady-state photolysis studies led to the identification of the water-insoluble 4,4′-dimeric, reduced pyridine compound formed by the dimerization of pyridinyl radicals. Quantum yields of $\mathrm{Pyr}^\bullet$ formation in the flash photolysis experiments were determined and compared with the quantum yields of $\mathrm{CO_2}$ formation and of Pyr^+ disappearance measured in the steady-state photolysis. A detailed mechanism for the pyridinium cation-sensitized photooxidation of sulfur-containing amino acids is proposed and discussed.

Introduction

The important role of excited states in biological processes[1,2] has stimulated numerous studies of the photochemical generation of radical centers in biologically relevant compounds. Photosensitization with aromatic ketones as the sensitizer has been used to induce electron- and hydrogen-transfer reactions in the flash and steady-state photolyses of amino acids[3−6] and proteins.[7] Moreover, since sulfur-containing amino acids serve key functions in biological systems,[8−10] a large effort has been made to understand the oxidation of the thioether moiety of these compounds. The effort has involved both the characterization of the reactive species in these oxidations and the detailed reaction mechanisms in which these species participate. Two complementary experimental approaches, i.e. pulse radiolysis[11,12] and laser flash photolysis, have been used to induce radical transformations involving sulfur-centered radicals and radical ions. Recently there have been detailed mechanistic investigations of 4-carboxybenzophenone (CB)-sensitized photooxida-

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[†] A. Mickiewicz University.

[‡] University of Notre Dame.

[§] On leave of absence from the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-532 Warsaw, Poland. Current address: Institute of Nuclear Chemistry and Technology, 03-195 Warsaw, Poland.

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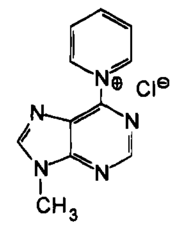
Chart 1



Pyr^+Cl^-

tions of sulfides,[13] sulfur-containing carboxylic acids,[14] and amino acids in aqueous solution.[15,16] The observation of ketyl radical anions, intermolecularly (S∴S)-bonded radicals cations, and intramolecularly (S∴O)-bonded species for some of the acids was direct evidence for the participation of electron transfer in the mechanism of quenching. Considering all the information gathered so far, the oxidation mechanism of sulfur-containing carboxylic acids and amino acids has revealed a very important irreversible route, namely, decarboxylation. The immediate chemical consequence of decarboxylation is the formation of α-(alkylthio)alkyl and α-aminoalkyl radicals, depending on the type of acid used. The presence of the latter radical, which is known to be a strongly reducing species,[17] is responsible for the dark reaction assigned to the one-electron reduction of the CB ground state.

The small exoergonicity of the electron-transfer process for the CB/sulfur-containing amino acids systems (for CB/methionione, $\Delta G = -18$ kJ/mol)[18] suggests searching for more efficient sensitizers. The triplet state of purinylpyridinium cations, e.g. *N*-(9-methylpurin-6-yl)pyridinium chloride, Pyr^+Cl^-, (Chart 1), should be an even better one-electron oxidant than the triplets of benzophenone derivatives in aqueous solution. In themselves, Pyr^+ cations are of biological significance as fluorescence probes in nucleic acids and other macromolecules[19–21] and as valuable intermediates in nucleoside chemistry.[22] Their known photophysical and photochemical properties,[20,21] their good solubility in water, and their potential as electron acceptors[21] make Pyr^+ cations excellent sensitizers for the photooxidation of sulfur-containing amino acids in aqueous solutions. In addition, the positive charge of the Pyr^+ molecule, contrary to the anionic character of CB previously studied,[15] permits an investigation of the interaction of a cationic sensitizer with quenchers in the zwitterionic (amino acid) or anionic (carboxylic acid) forms.

In this paper, we present and discuss the results of a detailed mechanistic study of the photosensitized oxidation of sulfur-containing amino acids by *N*-(9-methylpurin-6-yl)pyridinium cations in aqueous solution. The results provide data on the photoreactivity of the pyridinium salts (derived from the purine bases) in the presence of the electron donors such as sulfur-containing organic compounds and give new experimental evidence for the general mechanism of the sensitized photooxidation of thioethers proposed previously.[13]

Experimental Section

Materials. The sulfur-containing amino acids, 2,2′-thiodiethanoic acid, alanine, and ammonium acetate were obtained from Sigma and Fluka as the best available purity grades, and they were used without further purification. The pyridinium salt, *N*-(9-methylpurin-6-yl)pyridinium chloride was synthesized and purified by Dr. B. Skalski according to the procedure described previously.[23] Water was purified by a Millipore Milli-Q system.

Laser Flash Photolysis Experiments. The nanosecond laser flash photolysis has been described in detail elsewhere.[24] Laser excitation at 308 nm from a Lambda-Physik EMG 101 MSC (Xe/HCl) excimer laser (operated at 4–6 mJ, pulse width 20 ns) was used in a right-angle geometry of excitation. Rectangular quartz cells (0.5 × 1.0 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 double monochromator, a photomultiplier tube, and a pulsed xenon lamp of 1 kW as the monitoring source. The signal from the photomultiplier was processed by a 7912 AD Tektronix transient digitizer that was controlled by a LSI 11/2 microprocessor. Cutoff filters were used to avoid spurious response from second-order scattering from the monochromator gratings. The concentrations of sulfur-containing compounds were varied depending on the type of experiment. They were in the range 10^{-5}–10^{-4} M in the quenching experiments, but they were 1×10^{-2} M in the quantum yield determinations and in experiments for recording spectra in the time range following complete quenching of the Pyr^+ triplet state. In all these experiments the Pyr^+ concentrations were in the range of 2×10^{-4} M. For recording transient absorption spectra, the experiments were carried out with a continuous flow of the sample solution. The pHs of the solutions were adjusted to pH = 6.5 by adding sodium hydroxide. All solutions were deoxygenated by bubbling high-purity argon through them.

Steady-State Photolysis Experiments. Quantum Yield Determination. The irradiations were carried out on an optical bench irradiation system in a 1 × 1 cm rectangular cell. A high-pressure mercury lamp (HBO 200) with a combination of a BC-4 glass filter (Mashpriborintog, U.S.S.R.) and a 313 nm interference filter (Zeiss) was used for excitation. A solution of 2-hexanone in cyclohexane was used as an actinometer,[25] and the intensity of the incident light was determined to be 8.7×10^{-6} einstein dm^{-3} s^{-1}.

Solutions containing Pyr^+Cl^- (2×10^{-4} M) and various sulfur-containing amino acids (10^{-2} M) were adjusted with sodium hydroxide to pH 6.9, were purged with oxygen-free argon, and then were irradiated. The durations of irradiation times were chosen to cause about 10–15% of Pyr^+ conversion. The changes in the Pyr^+ concentrations during irradiation were determined spectroscopically using a Hewlett-Packard 8452A diode array spectrophotometer. In addition, these changes were monitored by HPLC. Before measurements, the dimer precipitate was removed by filtration using a membrane filter (0.45 mm). The HPLC analysis was performed on a Waters 600E system with a photodiode array detector using a reversed phase C4 column (Waters Delta Pack) eluted isocratically with 27.5% aqueous acetonitrile solution containing 0.1 M ammonium acetate.

Quantitative analysis of the CO_2 concentration was achieved using a gas chromatographic technique. The solutions after irradiations were acidified with concentrated HCl, and the vapor phase in the reaction cell was analyzed on a Chromatron GCHF gas chromatograph with a thermal conductivity detector on a Porapak Q column.

The determination of the quantum yield of Pyr^+ disappearance for the modified Stern–Volmer relation (eq 6) was performed with the Pyr^+ concentration equal to 2×10^{-4} M and the various methionine

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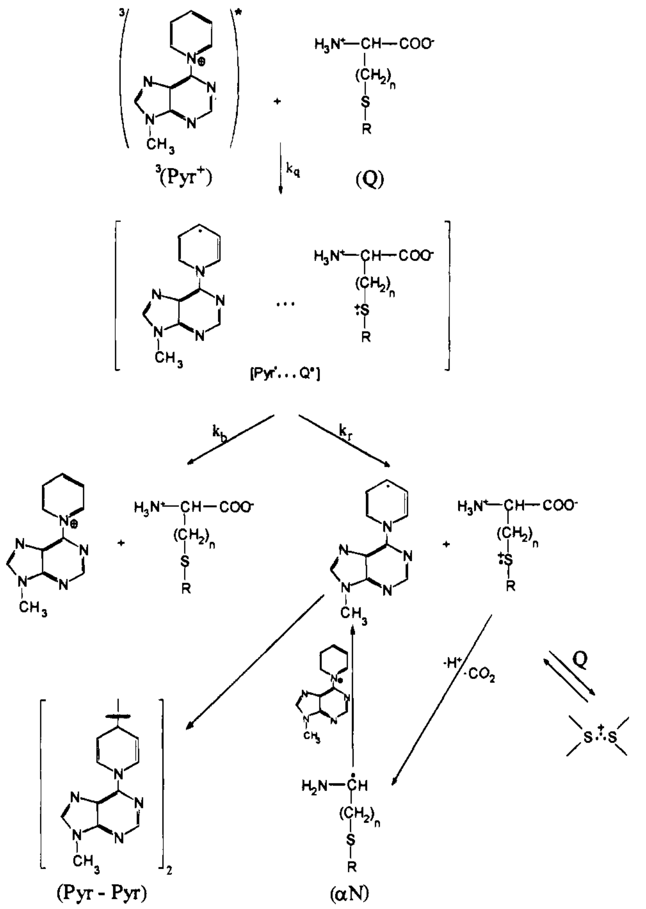
Scheme 1



concentrations in the range 10^{-5}–10^{-3} M. All experiments were performed at room temperature (22 ± 1 °C).

Preparative Irradiation. Preparation of the Pyr–Pyr Dimer. For preparative scale irradiations, an immersion apparatus (≈200 mL) equipped with an original Hanau TQ-150 high-pressure mercury lamp and a cylindrical Pyrex filter was used. An aqueous solution, that contained 2×10^{-3} M Pyr$^+$ salt and 2×10^{-2} M methionine, was adjusted to pH ≈7.0 with $NaHCO_3$, was deoxygenated by bubbling with oxygen free argon for about 30 min, and was then irradiated under a continuous flow of argon. Irradiation was continued until ≈60% of the Pyr$^+$ salt was reacted as revealed by UV analysis. The resulting suspension of a water-insoluble photoproduct was centrifuged. The solid material (red powder) was washed twice with water and dried under vacuum over P_2O_5. The photoproduct was identified as the 4,4'-dimeric, reduced pyridine compound (Pyr–Pyr dimer, Scheme 1) on the basis of ^{1}H NMR, IR, UV–visible, and MS analyses.

^{1}H NMR ($CDCl_3$, $T = 293$ K, Varian Gemini 300 spectrometer) (s, singlet; m, multiplet; Py, pyridine protons):

protons	chemical shifts in ppm (vs TMS)
2-H	8.48 s
8-H	7.26 s
Py-αH	8.23–8.21 m broad
Py-βH	5.16–5.13 m
Py-γH	3.26 s
N-CH₃	3.85 s

FT IR (IF-D 113v Bruker spectrometer, 1.3 mg/200 mg (substance/KBr)): 3083 cm^{-1} (C–H aromatic); 2950, 2922, 1452, 1365 cm^{-1} (C–H aliphatic); 1688 cm^{-1} (C=C); 1571 cm^{-1} (C=N); 1339, 1312, 1291 cm^{-1} (C–N).

UV (in CH_3CN solution, nm (ε)): 332 (max, 47 000 M^{-1} cm^{-1}), 258 (min, 5000 M^{-1} cm^{-1}).

EI MS (Jeol 90 FX spectrometer, 75 eV, *m/z* (relative intensity)): 424 (5, M$^+$), 212 (100), 133 (56).

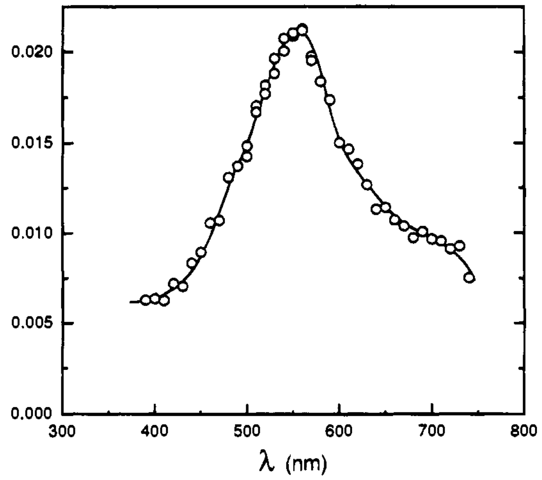


Figure 1. Spectrum of the triplet state of Pyr$^+$ obtained in the laser flash photolysis of an aqueous solution of Pyr$^+$ (2×10^{-4} M).

Pulse Radiolysis. Pulse radiolysis experiments were carried out at the Radiation Laboratory 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). Dosimetry was based on the N_2O-saturated thiocyanate dosimeter using a radiation chemical yield of 6.13 and an absorption coefficient of 7580 M^{-1} cm^{-1} at 472 nm for $(SCN)_2^{-}$.[26] Descriptions of the pulse radiolysis setup and data collection system are reported elsewhere.[27] The experiments were carried out with a continuous flow of the sample solution.

Electrochemical Experiments. The experiments were performed with an electrochemical analyzer from Bioanalytical Systems, model 100. The measurements were done with a standard three-component cell that contained a glass carbon working electrode, a platinum-wire counter electrode, and an SCE (saturated calomel) reference electrode. Cyclic voltammetric and square-wave voltammetric techniques were used. IR compensation was employed. To estimate the reduction potential of Pyr$^+$, voltammetric measurements were performed on acetonitrile or aqueous solutions (purged with nitrogen) of Pyr$^+$ perchlorate (1×10^{-3} M) with 0.1 M lithium perchlorate as the supporting electrolyte. The peak potentials obtained from the square-wave voltammetry were taken as the E_{red} for the Pyr$^+$ cation.

Results

A. Laser Flash Photolysis. Identification of the Pyr$^+$ Triplet State. The transient absorption spectrum obtained in the laser flash photolysis of the deoxygenated aqueous solution of Pyr$^+$ chloride (2×10^{-4} M) is presented in Figure 1. The long-lived absorption with the maximum at 550 nm (decay time ≈ 30 μs) was assigned to the triplet–triplet absorption of Pyr$^+$ on the basis of the following observations: (1) the absorption was quenched by oxygen with the rate constant of $k_q \approx 8 \times 10^8$ M^{-1} s^{-1}, i.e. in the range of one-ninth of the diffusion-controlled rate constant, (2) the absorption was efficiently quenched by sodium 2-anthracenesulfonate via an energy-transfer process ($k_q = (5.7 \pm 0.4) \times 10^9$ M^{-1} s^{-1}), and (3) the broad, long-lived transient absorption at 559 nm, observed for Pyr$^+$ in water by Skalski et al. in their picosecond transient absorption study,[21] had also been assigned to the triplet–triplet absorption.

The molar absorption coefficient of the triplet–triplet absorption of Pyr$^+$ in aqueous solution at 550 nm was determined to be $\epsilon = 8900$ M^{-1} cm^{-1} using the energy-transfer method and

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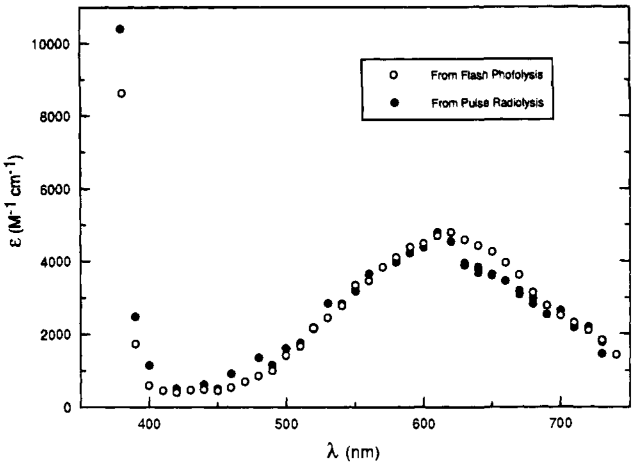

Figure 2. Transient absorption spectrum of the intermediate (Pyr•) observed during sensitized photooxidation of thiaproline by Pyr^+. ○○○ represents the spectrum obtained in the laser flash photolysis ($[Pyr^+] = 2 \times 10^{-4}$ M, [Thiaproline] = 0.01 M, pH = 6.5) at 15 μs after the flash. ●●● represents the spectrum obtained during the complementary pulse radiolysis experiments ($[Pyr^+] = 2 \times 10^{-4}$ M, pH = 6.1, N_2-saturated solution).

the procedure described in detail in ref 28. Sodium 2-anthracenesulfonate was used as a standard, taking the molar absorption coefficient at 425 nm to be $\epsilon_T = 3 \times 10^4$ M^{-1} cm^{-1}.[29]

The triplet yield of Pyr^+ was measured to be $\Phi_T = 0.38 \pm 0.07$ for an aqueous solution of Pyr^+ chloride (2×10^{-4} M) using the relative actinometry method[28] with 4-carboxybenzophenone in water as the actinometer ($\epsilon_T = 6250$ M^{-1} cm^{-1} at 535 nm).[30]

Transient Absorption Spectra of Intermediates. The aqueous solutions of Pyr^+ chloride and sulfur-containing amino acids ($[Pyr^+] = 2 \times 10^{-3}$ M, [quencher] = 0.02 M) were first examined spectroscopically for any evidence of ground-state association. The absorbance spectra of these mixtures were shown to be equal to those expected by adding the spectra from separate solutions of Pyr^+ and the quencher. No evidence for ground-state association was found under the experimental conditions used.

Flash excitation of the argon-purged solutions of Pyr^+Cl^- (2×10^{-4} M) and sulfur-containing amino acids (0.01 M) at pH = 6.5 resulted in the appearance of various, composite transient absorptions depending on the time delay and the structure of the amino acid used. High concentrations of quenchers were used to rapidly quench more than 99% of the Pyr^+ triplets (vide infra). For all quenchers, except for alanine (where only ≈15% quenching was observed), a new, long-lived transient absorption at 610 nm appeared in addition to the transient absorption band with the maximum at 550 nm associated with the Pyr^+ triplet. In the case of methionine, an additional short-wavelength shoulder in the region of 480 nm was also observed and assigned to the intermolecular (S∴S)-bonded radical cation of methionine.[15,31]

The transient absorption spectrum observed during the laser flash photolysis of Pyr^+Cl^- and thiaproline, taken 15 μs after the flash, consisted of a broad band with a maximum at 610 nm (Figure 2). The value of the molar absorption coefficient ($\epsilon_{610} = 5400 \pm 800$ M^{-1} cm^{-1}) was determined from the quenching of the Pyr^+ triplet by dimethylaniline (assuming electron-transfer quenching) in neutral aqueous solution, taking the molar absorption coefficient of dimethylaniline radical cation to be $\epsilon_{470} = 4500$ M^{-1} cm^{-1}.[32] The transient at 610 nm was tentatively assigned to the *N*-(9-methylpurin-6-yl)pyridinyl radical, Pyr•, as an expected product of the electron-transfer reaction from the sulfur atom of thiaproline (or from the nitrogen atom of dimethylaniline) to the Pyr^+ triplet state.

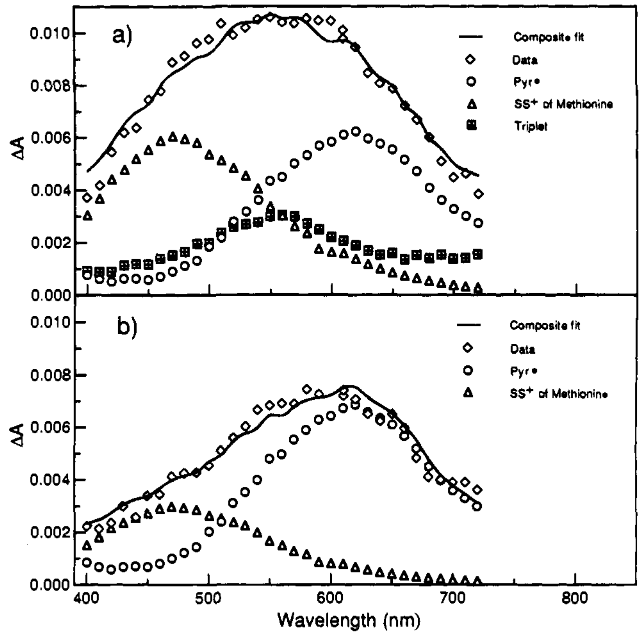

Figure 3. Resolution of the spectral components in the transient absorption spectra following the quenching of Pyr^+ triplet state in an aqueous solution of Pyr^+ (2×10^{-4} M) and methionine (0.010 M) at pH = 6.5 taken (a) at the end of the flash and (b) 24 μs after the flash. From the regression analysis the concentrations of transients were (a) 2.5×10^{-6} M, 1.6×10^{-6} M, and 6.7×10^{-7} M for Pyr•, $(S∴S)^+$ radical cation, and Pyr^+ triplet state, respectively, and (b) 3.0×10^{-6} M and 8.0×10^{-7} M for Pyr• and $(S∴S)^+$ radical cation, respectively.

The assignment of the transient at 610 nm to the Pyr• radical was supported by complementary pulse radiolysis experiments. The Pyr• radical was formed by the reaction of e_{aq}^- with Pyr^+ ($k_1 = (4.1 \pm 0.1) \times 10^{10}$ M^{-1} s^{-1}) according to

$$e_{aq}^- + Pyr^+ \rightarrow Pyr^\bullet \qquad (1)$$

The product of reaction 1 showed spectral characteristics similar to those of the long-lived transient in the above flash photolysis experiments ($\lambda_{max} = 610$ nm, $G\epsilon_{610} = 12\,720$ M^{-1} cm^{-1}) (Figure 2). Under experimental conditions of N_2-saturated aqueous solutions with 2×10^{-4} M pyridinium salt at pH 6.1, the radiation chemical yield of Pyr• radicals was equal to the radiation chemical yield of hydrated electrons (*G* = 2.65).[33] Division of $G\epsilon_{610}$ by the latter value yields the molar absorption coefficient $\epsilon_{610} = 4800$ M^{-1} cm^{-1}, which is slightly lower than that estimated in the laser flash photolysis experiment (vide supra).

The quantitative resolutions of the observed spectra, especially for methionine as a quencher of the Pyr^+ triplet state (Figure 3), were done using a linear multiple regression[34] of the form

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$$\Delta A(\lambda_j) = \sum_{i=1}^{n} \epsilon_i(\lambda_j) a_i \qquad (2)$$

where $\Delta A(\lambda_j)$ are the observed absorbance changes of the composite spectrum and $\epsilon_i(\lambda_j)$ are the molar absorption coefficients of the ith species at the jth wavelength of observation. Physically, the regression coefficients a_i are equal to $c_i l$ (where c_i is the concentration of the ith transient and l is the optical path length of the monitoring light). The details of the spectral resolution procedure have been described elsewhere.[18] The spectra of the Pyr$^+$ triplet and the Pyr$^\bullet$ radical were taken from this work, and the spectrum of the intermolecular (S∴S)-bonded radical cation of methionine was taken from refs 15 and 31. For the Pyr$^+$/methionine system, in addition to the Pyr$^+$ triplet state, two additional intermediates were identified from the composite spectrum (Figure 3a). The shoulder observed in the 480 nm region was assigned to the intermolecular (S∴S)-bonded radical cation. Such an intermediate was recently seen in the analogous systems, i.e. CB/sulfur-containing organic compounds.[14-16,18] The shoulder at about 610 nm in Figure 3a and a maximum in Figure 3b were identified as the absorbances due to the Pyr$^\bullet$ intermediate.

Quenching Rate Constants. The rate constants, k_q, for quenching of the triplet state of Pyr$^+$ by five sulfur-containing amino acids (i.e., methionine, thiaproline, *S*-methylcysteine, *S*-(carboxymethyl)cysteine, and lanthionine), 2,2′-thiodiethanoic acid, alanine (a non-sulfur-containing amino acid), and ammonium acetate were determined in aqueous solution at pH = 6.5. Since only neutral solutions were studied, it will be understood that the amino acids were in their zwitterionic form,[35] with the exception of *S*-(carboxymethyl)cysteine and that 2,2′-thiodiethanoic acid was in its ionic form.[14,15]

The k_q values were obtained by monitoring the triplet–triplet absorption decays of Pyr$^+$ at fixed wavelengths for various quencher concentrations and by employing the Stern–Volmer relation

$$k_{obs} = (\tau_T)^{-1} + k_q[Q] \qquad (3)$$

where τ_T is the lifetime of the Pyr$^+$ triplet in the absence of quencher.

A typical experimental trace for the triplet decay in the presence of 8×10^{-5} M thiaproline is presented in the insert of Figure 4. The long-lived absorption has been attributed to the formation of Pyr$^\bullet$ (vide supra). The higher ratio of molar absorption coefficients of triplet vs Pyr$^\bullet$ absorption at 440 nm compared to the corresponding ratio at 550 nm (maximum of the T–T absorption) made 440 nm a more favorable monitoring wavelength for triplet decays.

The pseudo-first-order rate constants, k_{obs}, were calculated using eq 4, which takes into account a concomitant, underlying first-order growth of the photoproduct's absorption[36]

$$\ln\left(\frac{A - A^\infty}{A^0 - A^\infty}\right) = -k_{obs}t \qquad (4)$$

where A^0, A, and A^∞ are the absorbance changes at time 0, t, and infinity, respectively. A typical plot based on eq 3 is presented in Figure 4, and the quenching rate constants obtained for all the quenchers used are summarized in Table 1.

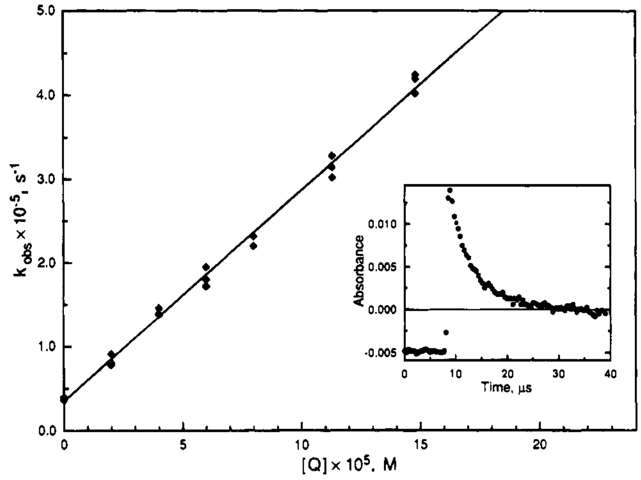

Figure 4. Stern–Volmer plot for the quenching of Pyr$^+$ triplet state by thiaproline in aqueous solution at pH = 6.5. Insert: kinetic trace for the Pyr$^+$ triplet state decay at 440 nm in the presence of 8×10^{-5} M thiaproline ([Pyr$^+$] = 2×10^{-4} M).

Secondary Reactions of the Pyr$^+$ Cation Ground State. At high concentrations of amino acids (thiaproline and methionine) such that the triplet state of Pyr$^+$ is quenched within a short time (<100 ns) after the laser pulse, the experimental traces measured at 610 nm clearly indicated two processes of Pyr$^\bullet$ formation: a fast component that occurred on a nanosecond time scale and a slow one that occurred on a microsecond time scale. The latter process was characterized by pseudo-first-order rate constants, which depended linearly on the pyridinium cation ground-state concentration. A typical experimental trace observed at λ = 610 nm (fast and slow components of Pyr$^\bullet$ radical formation) for thiaproline is presented in the upper insert of Figure 5. This slow growth is not observed for *S*-(carboxymethyl)cysteine or *S*-methylcysteine (vide infra); the solubility of lanthionine in water was too low to totally quench the Pyr$^+$ in this type of experiment. A linear dependence of the pseudo-first-order rate constants on the Pyr$^+$ ground-state concentration is shown in Figure 5. The slope of this plot gives the bimolecular rate constant ($k = (2.85 \pm 0.12) \times 10^9$ M^{-1} s^{-1}) for the one-electron reduction of the Pyr$^+$ ground state in the Pyr$^+$/thiaproline system.

A typical decay of the 610 nm absorbance, shown in the lower insert of Figure 5, was described as a second-order reaction with the rate constant estimated to be $2k_2 = 2 \times 10^9$ M^{-1} s^{-1}. The reaction is likely due to the recombination of Pyr$^\bullet$ radicals to form a Pyr–Pyr dimer (vide infra).

Quantum Yields of Pyr$^\bullet$ and Intermolecular (S∴S)$^+$ Dimer Formation. The quantum yields of the formation of transients (Pyr$^\bullet$ and (S∴S)$^+$ dimer) were determined for thiaproline and methionine using large concentrations of the amino acids so that the Pyr$^+$ triplets were almost totally quenched (>99%). The flash photolysis experiments were carried out in matched optically flat cells with identical absorbances at 308 nm due to the Pyr$^+$ and the 4-carboxybenzophenone (used as an actinometer) ground-state absorptions. For Pyr$^+$/amino acid systems, the absorption changes at the spectral maxima of the Pyr$^\bullet$ radical and the (S∴S)-bonded radical cation intermediate were measured and compared with an absorption change immediately after the flash at 535 nm due to the 4-carboxybenzophenone triplet. The quantum yields were calculated according to eq 5, where ΔA_P is the product's

$$\Phi = \frac{\Delta A_P \epsilon_T}{\Delta A_T^{\,0} \epsilon_P} \qquad (5)$$

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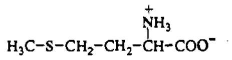
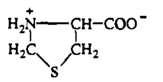
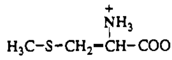
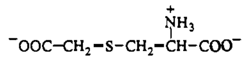
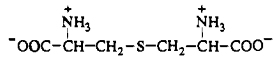
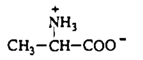
[35] Lehninger, A. L. *Biochemie*; Verlag Chemie: Weinheim, Germany, 1985.

[36] Encinas, M. V.; Wagner, P. J.; Scaiano, J. C. *J. Am. Chem. Soc.* **1980**, *102*, 1357–1360.

Table 1. Rate Constants for Quenching of the Pyr$^+$ Triplet State in Aqueous Solution at pH = 6.5 ([Pyr$^+$] = 2 × 10^{-4} M)

quencher	formula	$k_q \times 10^{-9}$ (M^{-1} s^{-1})^a
methionine	 $H_3C{-}S{-}CH_2{-}CH_2{-}CH(\overset{+}{N}H_3){-}COO^-$	2.15 ± 0.20
thiaproline		2.55 ± 0.14
S-methylcysteine	 $H_3C{-}S{-}CH_2{-}CH(\overset{+}{N}H_3){-}COO^-$	1.82 ± 0.08
S-(carboxymethyl)cysteine	 $^-OOC{-}CH_2{-}S{-}CH_2{-}CH(\overset{+}{N}H_3){-}COO^-$	3.60 ± 0.20
lanthionine	 $^-OOC{-}CH(\overset{+}{N}H_3){-}CH_2{-}S{-}CH_2{-}CH(\overset{+}{N}H_3){-}COO^-$	0.21 ± 0.02
2,2′-thiodiethanolic acid	$^-OOC{-}CH_2{-}S{-}CH_2{-}COO^-$	7.8 ± 0.3
alanine	 $CH_3{-}CH(\overset{+}{N}H_3){-}COO^-$	~6 × 10^{-4}
ammonium acetate	$CH_3{-}COO^-$	(3.6 ± 0.5) × 10^{-3}

^{a} Errors taken as twice the standard deviations from the least-squares fits.

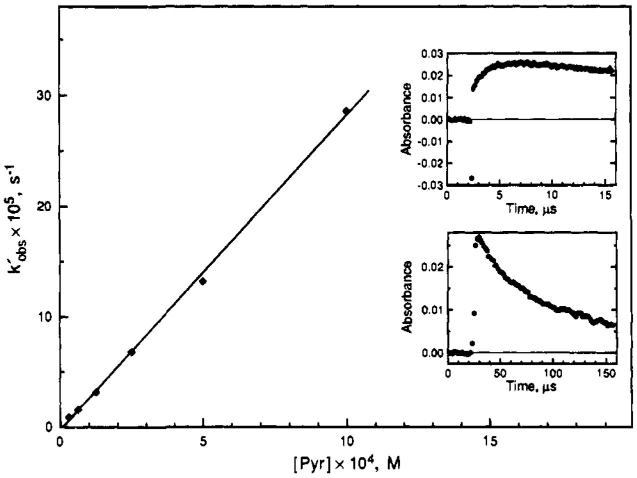


Figure 5. Pyr$^+$ concentration dependence of the pseudo-first-order rate constant, k'_{obs}, for the reaction of Pyr$^+$ ground state with α-aminoalkyl radicals derived from thiaproline. Inserts: kinetic traces for growth and decay of Pyr$^•$ absorbance observed at 610 nm ([Pyr$^+$] = 2 × 10^{-4} M, [thiaproline] = 5 × 10^{-3} M).

absorption change extrapolated either (a) to the "end-of-pulse" value under the conditions of nearly complete quenching or (b) to a maximum value (including a correction for the decay of the 610 nm absorption) corresponding to the total formation of Pyr$^•$, i.e. in the photochemical and secondary processes (see the upper insert in Figure 5). $\Delta A_T{}^0$ is the absorption change immediately after the flash due to the 4-carboxybenzophenone triplet (actinometer) at 535 nm measured under the conditions of no quenching. The molar absorption coefficients (ε) of the Pyr$^•$ radical were taken as ϵ_p(610 nm) = 4800 M^{-1} cm^{-1} and ϵ_p(480 nm) = 850 M^{-1} cm^{-1} (vide supra), the values for ϵ_p of the (S∴S)$^+$ dimer of methionine as ϵ_p(610 nm) = 1930 M^{-1} cm^{-1} and ϵ_p(480 nm) = 7300 M^{-1} cm^{-1}),[15,31] and the value for the 4-carboxybenzophenone triplet as ϵ_T(535 nm) = 6250 M^{-1} cm^{-1}.[30] Since the absorption spectra of Pyr$^•$ and the (S∴S)$^+$ dimer of methionine overlap, the absorption change at λ_{max} of the particular species was corrected to remove the contribution from the remaining species. The values of the quantum yields reached by this method for Pyr$^•$ and the (S∴S)-bonded radical cation of methionine are summarized in Table 2. $\Phi_{Pyr^•}$ represents the quantum yield of Pyr$^•$ formation in the primary photochemical reaction, and $\Phi'_{Pyr^•}$ is the sum of quantum yields of the photochemical and the secondary reactions (the total quantum yield of Pyr$^•$ formation). The values of the quantum yields (Table 2) obtained using the above procedure are in good agreement with those determined on the basis of the concentrations of appropriate intermediates estimated from the resolution of the spectral components in the absorption spectra taken at appropriate time delays (Figure 3).

B. Steady-State Photolysis. Identification of the Pyr–Pyr Dimer. The preparative scale irradiation of a deoxygenated aqueous solution, adjusted to pH ≈7.0, containing 2 × 10^{-3} M Pyr$^+$ Cl$^-$ and 2 × 10^{-2} M methionine led to the formation of an insoluble product in water. The structure of the product was established as the 4,4′-dimeric, reduced pyridine compound (Scheme 1) on the basis of ^{1}H NMR spectra (see the table with the NMR spectral assignments in the Experimental Section). This was additionally supported by the results of an MS analysis and IR and UV–visible spectra. The appearance of the molecular ion and the fragmentation pattern in the MS spectrum clearly confirmed the dimeric structure of the photoproduct obtained.

Quantum Yields of Pyr$^+$ Disappearance and CO_2 Formation. Supplementary measurements of quantum yields of Pyr$^+$ disappearance, Φ_{Pyr^+}, and CO_2 formation, Φ_{CO_2}, were performed in steady-state photolysis experiments. All quantum yields obtained were the average of at least three independent measurements and are summarized in the last two columns of Table 2. The values of Φ_{Pyr^+} from the steady-state determinations agree well with the values of $\Phi'_{Pyr^•}$ obtained by flash photolysis (Table 2).

The determination of the quantum yield of Pyr$^+$ disappearance was also performed for various methionine concentrations to confirm the expected linear dependence of $1/\Phi_{Pyr^+}$ vs the reciprocal of the amino acid concentration (eq 6). The results obtained are presented in Figure 6.

Discussion

In order to study the interaction of the Pyr$^+$ triplet state with sulfur-containing amino acids, the properties of the Pyr$^+$ triplet state in aqueous solution were first characterized using nanosecond flash photolysis. A transient absorption band with a maximum at 550 nm and ϵ_{max} = 8.9 × 10^3 M^{-1} cm^{-1} was assigned to the $T_1 \rightarrow T_n$ absorption (decay time of $\tau_T \approx 30$ µs). The large value of the intersystem crossing quantum yield (Φ_T = 0.38 ± 0.07), in spite of a large value of the fluorescence

Table 2. Quantum Yields of $\mathrm{Pyr}^\bullet$ Radical Formation ($\Phi_{\mathrm{Pyr}^\bullet}$ and $\Phi'_{\mathrm{Pyr}^\bullet}$), Pyr^+ Disappearance (Φ_{Pyr^+}), and CO_2 Formation (Φ_{CO_2})

	flash photolysis		steady-state photolysis	
quencher	$\Phi_{\mathrm{Pyr}^\bullet}$	$\Phi'_{\mathrm{Pyr}^\bullet}$	Φ_{Pyr^+}	$\Phi_{\mathrm{Pyr}^+}/\Phi_{CO_2}$ [c]
thiaproline	0.31 ± 0.03	0.63 ± 0.06	0.62 ± 0.05	1.8 (2.2−1.5)
methionine	*a*	0.27 ± 0.03[b]	0.20 ± 0.03	2.5 (3.4−1.9)
S-(carboxymethyl)cysteine	*a*	*a*	0.18 ± 0.02	1.2 (1.6−0.9)
2,2′-thiodiethanoic acid	*a*	*a*	0.15 ± 0.03	1.1 (1.4−0.9)

[a] Not measured. [b] The quantum yield of $(\mathrm{S}\therefore\mathrm{S})^+$ dimer formation was determined to be $\Phi_{\mathrm{SS}^+} = 0.20$. [c] The ratio of quantum yields of Pyr^+ disappearance and CO_2 formation (in parentheses, the range of quantum yield ratio obtained on the basis of the experimental errors of $[\mathrm{Pyr}^+]$ and $[CO_2]$ determinations).

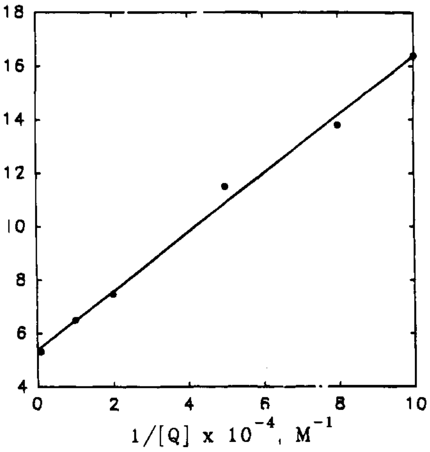


Figure 6. Plot of $1/\Phi_{\mathrm{Pyr}^+}$ vs the reciprocal of methionine concentration in aqueous solutions at pH = 6.9 (from steady-state photolysis).

quantum yield ($\Phi_f = 0.46 \pm 0.05$),[20] indicates that Pyr^+ can be used as a triplet sensitizer.

The quenching of the Pyr^+ triplet state was shown to occur via electron transfer from the sulfur atom of the amino acid to the triplet state of Pyr^+. This conclusion was supported by the following arguments: (1) direct observation of electron-transfer intermediates, i.e. the $\mathrm{Pyr}^\bullet$ radical and the intermolecular $(\mathrm{S}\therefore\mathrm{S})$-bonded radical cation of some of the sulfur-containing amino acids, (2) the formation of the Pyr−Pyr dimer as a stable photoproduct (in addition to the second-order decay of the $\mathrm{Pyr}^\bullet$ absorbance at 610 nm), (3) the exoergonicity of the electron-transfer process ($\Delta G_{\mathrm{el}} \approx -100$ kJ/mol, vide infra), (4) the large values of the quenching rate constants that are similar to those for electron-transfer quenching of the 4-carboxybenzophenone triplet state by sulfur-containing amino acids, and (5) the k_q values for alanine (a non-sulfur-containing amino acid) and ammonium acetate, which are 3−4 orders of magnitude lower than those for quenchers containing the thioether moiety.

On the basis of the experimental results, we propose a mechanism for the Pyr^+ triplet-sensitized photooxidation of sulfur-containing amino acids in aqueous neutral solutions (Scheme 1). The initial step in the quenching mechanism is an electron transfer from a sulfur atom to the Pyr^+ triplet state, creating a CT complex (ion radical pair), $[\mathrm{Pyr}^\bullet...\mathrm{Q}^+]$ (Scheme 1). The CT complex can disappear by (1) diffusion apart to form a sulfur-centered radical cation ($>\mathrm{S}^{\bullet+}$) and a $\mathrm{Pyr}^\bullet$ radical or (2) back electron transfer to regenerate the reactants in their ground states. Further secondary reactions of the intermediates (Scheme 1) depend on the structure and concentration of the amino acids used and on the concentration of the Pyr^+ salt. The secondary reactions consist of a decarboxylation of the sulfur-centered radical cation to form an α-aminoalkyl radical (αN), followed by a further one-electron reduction of Pyr^+ by an αN radical and a reaction of an $>\mathrm{S}^{\bullet+}$ radical cation with the amino acid to form an $(\mathrm{S}\therefore\mathrm{S})^+$ dimer. The results of flash photolysis (e.g., Figure 5, Table 2) and steady-state photolysis experiments (detection of CO_2 and the Pyr−Pyr dimer, Table 2, Figure 6) confirm the reaction pattern presented in Scheme 1.

The rate constants, k_q, for quenching of the Pyr^+ triplet state by sulfur-containing amino acids (in their zwitterionic form) are in the range $(1.8{-}2.6) \times 10^9$ M^{-1} s^{-1} and are similar to those for quenching of the 4-carboxybenzophenone triplet in aqueous solution.[15] The k_q values for the sulfur-containing quenchers in their anionic forms, increase in the sequence $k_q(\mathrm{Q}^0) < k_q(\mathrm{Q}^{-1}) < k_q(\mathrm{Q}^{-2})$, as expected for quenching of a cationic species by charged quencher molecules.[37] Similar behavior was recently observed for quenching of the 4-carboxybenzophenone triplet by non-amino sulfur-containing carboxylic acids.[14] The effect of reactant charges on the quenching rate constants of Pyr^+ fluorescence by various sulfur-containing organic compounds in aqueous solution will be discussed quantitatively elsewhere.[38]

On the other hand, for alanine and ammonium acetate, which lack a sulfur moiety, the k_q's are at least 3−4 orders of magnitude lower, respectively, than the k_q's obtained for sulfur-containing compounds. This observation further supports the idea that the quenching of the $^3\mathrm{Pyr}^+$ state by sulfur-containing organic compounds involves an electron transfer from the sulfur atom.

The standard free energy change for electron transfer (the driving force for the process) was estimated as $\Delta G_{\mathrm{el}} = -107$ kJ/mol from the Weller equation,[39] $\Delta G_{\mathrm{el}} = F(E_{\mathrm{ox}} - E_{\mathrm{red}}) - E_T + \Delta w$. This value for ΔG_{el} resulted from taking the oxidation potential for methionine to be $E_{\mathrm{ox}} = 1.1$ V,[18] the reduction potential of the Pyr^+ cation to be $E_{\mathrm{red}} = -0.57$ V (see the Experimental Section), the energy of the triplet state of Pyr^+ to be $E_T = 268$ kJ/mol,[20] and the Δw term, describing the work required to bring the products and reactants from infinity to the reaction radius in the complex, as $\Delta w = 0$. The ΔG_{el} value obtained shows that there is about five times the driving force for the Pyr^+ triplet/methionione system than for the 4-carboxybenzophenone triplet/methionine system ($\Delta G_{\mathrm{el}} = -18$ kJ/mol). In spite of this much more negative ΔG_{el} for the Pyr^+ triplet/methionine system than for the 4-carboxybenzophenone triplet/methionine system, the k_q values are the same within experimental error. This can be explained in terms of a Rehm−Weller plot in the electron-transfer quenching presented recently for quenching of substituted benzophenone triplets by methionine,[18] where for exoergonic electron-transfer reactions with $\Delta G_{\mathrm{el}} < -15$ kJ/mol, the plateau region was reached with k_q in the range of 2×10^9 M^{-1} s^{-1}.

A secondary photoreaction process was directly observed on the microsecond time scale in the flash photolysis experiments with the quenchers thiaproline and methionine (upper insert in Figure 5). This process was assigned to the one-electron

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

(38) Marciniak, B.; Rozwadowski, J.; Bobrowski, K. *J. Photochem. Photobiol., A.* To be submitted.

(39) Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259−271.

reduction of the Pyr$^+$ ground state by the α-aminoalkyl radicals produced in the decarboxylation of the sulfur-centered radical cation (Scheme 1). The absence of this reaction with the quencher *S*-(carboxymethyl)cysteine was explained by the efficient decarboxylation of the carboxyl group located on the carbon α to the sulfur. The resulting α-(alkylthio)alkyl radicals have weaker reducing properties than α-aminoalkyl radicals, which are strong reducing agents.[17] This is supported by the absence of secondary photoreaction processes in the CB/2-(methylthio)ethanoic acid and the CB/2,2′-thiodiethanoic acid systems, where the decarboxylation led to the formation of α-(alkylthio)alkyl radicals.[14] The lack of an observation of a secondary reaction for *S*-methylcysteine is due to the short lifetime of the α-aminoalkyl radicals derived from this amino acid.[15] Since the absorption spectra of Pyr$^•$ and (S∴S)$^+$ dimers of methionine overlap, for simplicity, the secondary reaction was quantitatively studied for thiaproline, which does not form (S∴S)$^+$ dimers. The linear dependence of these pseudo-first-order rate constants on the pyridinium cation ground-state concentration (Figure 5) permitted a determination of the bimolecular rate constant for the one-electron reduction of the Pyr$^+$ ground state by α-aminoalkyl radicals produced from thiaproline ($k = (2.85 \pm 0.12) \times 10^9$ M^{-1} s^{-1}). This value is about three times higher than that for the reduction of the ground state of 4-carboxybenzophenone by thiaproline ($k = 0.93 \times 10^9$ M^{-1} s^{-1}).[15] This is in agreement with the change of the values of the reduction potential in going from Pyr$^+$ ($E_{\rm red} = -0.57$ V) to 4-carboxybenzophenone ($E_{\rm red} \approx -1.8$ V).[18]

On the basis of Scheme 1, using the steady-state approximation, the following linear expression relating the reciprocal of the quantum yield of Pyr$^+$ disappearance ($1/\Phi_{\rm Pyr^+}$) to the reciprocal of the quencher concentration can be derived (modified Stern−Volmer relation):

$$\frac{1}{\Phi_{\rm Pyr^+}} = \frac{1}{\Phi_{\rm Pyr^+}^{\rm lim}}\left(1 + \frac{1}{k_{\rm q}\tau_{\rm T}[{\rm Q}]}\right) \qquad (6)$$

where $\Phi_{\rm Pyr^+}^{\rm lim}$ is the limiting quantum yield of Pyr$^+$ disappearance (describing the case where the Pyr$^+$ triplet state is totally quenched due to the high quencher concentrations used) and $\tau_{\rm T}$ is the lifetime of the Pyr$^+$ triplet in the absence of quencher. The results obtained for methionine presented in Figure 6 show, as expected from eq 6, a linear dependence of $1/\Phi_{\rm Pyr^+}$ vs 1/[Q]. This permitted the determination of the quenching rate constants from the steady-state measurements, $k_{\rm q} = (1.9 \pm 0.4) \times 10^9$ M^{-1} s^{-1}. This value is in good agreement with the quenching rate constant obtained in the flash photolysis experiments according to eq 1 (Table 1). This is an additional justification of the mechanism proposed in Scheme 1.

In the case of thiaproline, the quantum yield of Pyr$^•$ radical formation, $\Phi'_{\rm Pyr^•}$, which is equal to 0.63 (Table 2), consists of two terms. The first represents the formation of Pyr$^•$ in the primary photochemical process ($\Phi_{\rm Pyr^•} = 0.31$), and the second term ($\Phi''_{\rm Pyr^•} = 0.32$) is due to the secondary reduction of the Pyr$^+$ ground state by the α-aminoalkyl radicals produced in the decarboxylation of the sulfur-centered radical cation (Scheme 1). This clearly indicates that the secondary reactions are 100% efficient under the experimental conditions used. Two supplementary, independent steady-state measurements further support this conclusion: (1) the value of the Pyr$^+$ disappearance was determined to be $\Phi_{\rm Pyr^+} = 0.62$, and (2) the ratio $\Phi_{\rm Pyr^+}/\Phi_{\rm CO_2}$ was found to be ≈2. The values of the intersystem crossing quantum yield for Pyr$^+$ ($\Phi_{\rm T} = 0.38$) and $\Phi_{\rm Pyr^•} = 0.31$ for the radical production by thiaproline permitted an estimation of the contribution of the diffusion apart reaction of the CT complex (82%) in comparison to the back electron transfer process (18%) (reactions $k_{\rm r}$ and $k_{\rm b}$ in Scheme 1). These numbers clearly indicate that the diffusion apart (escape) is the main process for the fate of the CT complex in the quenching events of ^{3}Pyr$^+$ and thiaproline. The formation of (S∴S)$^+$ dimers in the case of methionine did not permit as accurate a quantum yield determination as for thiaproline. However, the results obtained for methionine (Table 2) also suggest that the diffusion apart reaction is an efficient (≈50%) pathway for the CT complex disappearance. A similar conclusion can be also drawn on the basis of the values of quantum yields ($\Phi_{\rm Pyr^+}$ and $\Phi_{\rm Pyr^+}/\Phi_{\rm CO_2}$) determined in the steady-state photolysis experiments for *S*-(carboxymethyl)cysteine and 2,2′-thiodiethanoic acid (Table 2).

Conclusions

It was shown that transfer of an electron from the sulfur atom to the triplet state of Pyr$^+$ was a primary step in the photosensitized oxidation of sulfur-containing amino acids. This was established by the direct observation of electron-transfer intermediates in the transient absorption spectra, i.e. Pyr$^•$ and the intermolecular (S∴S)-bonded radical cation (for methionine), large values of the rate constants ($k_{\rm q}$) for quenching of the Pyr$^+$ triplet state by sulfur-containing amino acids (in the range of 2×10^9 M^{-1} s^{-1}), in contrast to 4 orders of magnitude lower $k_{\rm q}$ values for alanine (a non-sulfur-containing amino acid), and detection of Pyr−Pyr dimers in the steady-state photolysis.

The electron-transfer process leading to the formation of the CT complex, [Pyr$^•$...Q$^+$], is followed by (1) escape of the Pyr$^•$ (a fast formation of Pyr$^•$ on the nanosecond time scale) from the sulfur-centered radical cation and (2) the back electron transfer to regenerate the reactants in their ground states. The contribution of escape was shown to be a dominant pathway (82%) for thiaproline and was found to be about 50% for other amino acids investigated.

The slow formation of Pyr$^•$ radicals, which occurred on a microsecond time scale, was assigned to the one-electron reduction of the pyridinium cation ground state by the α-aminoalkyl radicals produced from the decarboxylation of the sulfur-centered radical cations.

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