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Quenching of the excited singlet state of the *N*-(9-methylpurin-6-yl)pyridinium cation by sulphur-containing amino acids and carboxylic acids in aqueous solution

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Abstract

Quenching of the lowest excited singlet state of the *N*-(9-methylpurin-6-yl)pyridinium cation (Pyr^+) by sulphur-containing organic compounds in neutral aqueous solution was studied by steady state and time-resolved fluorescence techniques. The quenching rate constants k_q were determined from the Stern–Volmer plots for several sulphur-containing amino acids and carboxylic acids (Q^{-Z_B}) in their zwitterionic ($Z_B=0$) and anionic ($Z_B=-1$, -2 and -3) forms on the basis of lifetime and fluorescence intensity measurements at constant ionic strength (μ). The rate constants (extrapolated to $\mu=0$) for the dynamic quenching process were found to be in the range $(0.4–4)\times10^{10}$ $M^{-1}\,s^{-1}$ depending on the ionic charge Z_B, and were close to the diffusion-controlled limit. For comparison, analogous rate constants measured for alanine, glycine and proline (non-sulphur amino acids) were found to be at least four times lower, $k_q=1\times10^9$ $M^{-1}\,s^{-1}$. The ion pair association constants, corresponding to the static quenching constants obtained by combining the intensity and lifetime quenching data, were in the range 5–11 M^{-1}, indicating that the observed quenching was mainly due to a dynamic process. The mechanism of quenching was discussed in terms of electron transfer from the sulphur atom of Q^{-Z_B} to the Pyr^+ singlet state, and was compared with the quenching of the Pyr^+ triplet state recently shown to occur via the electron transfer process (*J. Am. Chem. Soc., 117* (1995) 127).

Keywords: Electron transfer; Fluorescence quenching; Pyridinium cation; Singlet states; Sulphur-containing amino acids and carboxylic acids

1. Introduction

The biological importance of electron transfer reactions induced by the excited states of various organic sensitizers [1], with the participation of the sulphide function in sulphur-containing peptides and proteins [2], requires a thorough understanding of the mechanism of photoinduced electron transfer in less complicated systems [3]. Recently, detailed mechanistic investigations of the 4-carboxybenzophenone-sensitized (CB-sensitized) photo-oxidation of thioethers [4], sulphur-containing carboxylic acids [5] and amino acids [6–8] and methionine-containing dipeptides and tripeptides [9] in aqueous solution have been performed. For all these systems, electron transfer from the sulphur atom to the triplet state of the ketone was found to be the primary photochemical step. The observation of a ketyl radical anion $CB^{\cdot-}$, intermolecularly (S∴S)-bonded radical cation and intramolecularly (S∴O)-bonded and (S∴N)-bonded species provided direct evidence for the electron transfer process. Further secondary photoreactions were shown to be very rich (e.g. decarboxylation, reduction of CB by α-aminoalkyl radicals, protonation), and varied with the structure of the sulphur-containing quencher and with the experimental conditions employed [3].

The small exoergonicity of the electron transfer reaction for the CB–sulphur-containing amino acid system ($\Delta G_{el}=-18$ kJ mol^{-1} [10]) suggested a search for more powerful oxidants. Recently, the *N*-(9-methylpurin-6-yl)pyridinium cation (Pyr^+) in the triplet state has been used as a one-electron oxidant in the sensitized photo-oxidation of sulphur-containing compounds [10]. Its known photophysical properties [11,12] (see Fig. 1), the reduction potential ($E_{red}=-0.57$ V [11]) and the good solubility of Pyr^+ salts in water make the Pyr^+ cation an excellent triplet sensitizer for the photo-oxidation of sulphur-containing amino acids ($\Delta G_{el}=-100$ kJ mol^{-1} [11]). The electron transfer from the sulphur atom to the Pyr^+ triplet state was established by the direct detection of electron transfer intermediates, i.e. $Pyr^{\cdot}$ radical and the intermolecularly (S∴S)-bonded radical cation. Secondary photoreactions were found to be similar to

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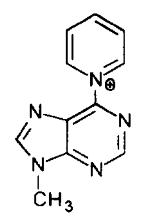


(Pyr^+)

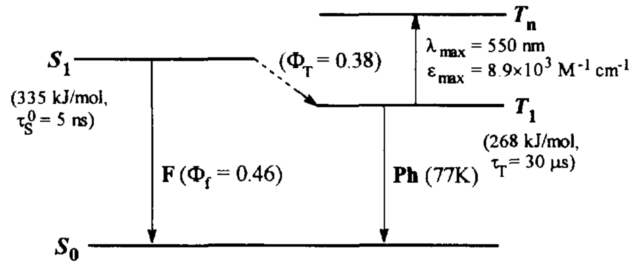


Fig. 1. Jablonski diagram for Pyr^+ cation in aqueous solution (data taken from Refs. [11,12]).

those for the CB–sulphur-containing amino acid system, e.g. one-electron reduction of the pyridinium cation ground state by the α-aminoalkyl radical produced from the decarboxylation of the sulphur-centred radical cation [11].

The biological significance of pyridinium cations (fluorescence probes in nucleic acids and other macromolecules [12]) provides an additional argument for the study of the interaction of the excited singlet state of Pyr^+ with sulphur-containing organic compounds. In this paper, we present and discuss the quenching of the Pyr^+ fluorescence by sulphur-containing amino acids and carboxylic acids in aqueous solution.

2. Experimental details

2.1. Materials

The sulphur-containing amino acids and carboxylic acids (alanine, glycine and proline) were obtained from Sigma, Fluka and Aldrich as the best purity grade available, and were used without further purification (except 3-carboxymethylthiopropionic acid which was recrystallized twice from water). The sample of pyridinium salt, *N*-(9-methylpurin-6-yl)pyridinium chloride, was a generous gift from Dr. B. Skalski (Adam Mickiewicz University, Poznań, Poland). Water was purified by a Millipore Milli-Q system.

2.2. Solutions

In the quenching experiments, the concentrations of sulphur-containing compounds were in the range $(0.5–3)\times10^{-2}$ M and those of non-sulphur-containing amino acids were in the range $(0.5–5)\times10^{-2}$ M; the pyridinium salt concentration was 2×10^{-5} M. In the photochemical and flash photolysis studies, the concentration of pyridinium salt was 2×10^{-4} M and that of methionine was 0.2 M (2,2′-thiodiethanoic acid, 1 M). The pH and ionic strength of the solution were adjusted by adding NaOH and $NaClO_4$.

2.3. Methods

The UV–visible absorption spectra were recorded with a Hewlett-Packard 8542 diode array spectrophotometer, and the steady state fluorescence spectra were recorded using a Perkin–Elmer MPF 66 spectrofluorometer. Fluorescence lifetime measurements were performed using the time-correlated single-photon counting technique with an IBH Consultants fluorescence lifetime system (model 5000).

Fluorescence quenching experiments were performed by measuring the intensities of the characteristic Pyr^+ emission at $\lambda_f = 525$ nm (excitation wavelength, $\lambda_{ex} = 300$ nm) and the lifetimes at room temperature (20 ± 1 °C). Solutions for quenching experiments were not deoxygenated and thus all results refer to air-saturated solutions.

The photochemical experiments were carried out in a 1 cm × 1 cm rectangular UV cell on an optical bench irradiation system. A high-pressure mercury lamp (HBO 200), with a combination of a BC-4 glass filter (Mashpriborintog) and a 313 nm interference filter (Zeiss), was used for excitation. A solution of 2-hexanone in cyclohexane was used as actinometer [13], and the intensity of the incident light was determined to be 4.7×10^{-5} einstein dm^{-3} s^{-1}. Prior to irradiation, the solutions were deoxygenated by bubbling with oxygen-free argon for about 15 min. The changes in the Pyr^+ concentration during irradiation were determined spectroscopically.

The flash photolysis test experiments were performed using a nanosecond laser flash photolysis system at the Radiation Laboratory, University of Notre Dame. Details of this apparatus are given in Ref. [11]. All solutions were deoxygenated by bubbling with high-purity argon.

3. Results and discussion

In previous studies, we have investigated the interaction of the triplet state of the Pyr^+ cation with sulphur-containing amino acids in aqueous solution [11]. We have shown that the transfer of an electron from the sulphur atom to the triplet state of Pyr^+ is the primary step in the photosensitized oxidation of sulphur-containing organic compounds. The rate constants of quenching of the Pyr^+ triplet state by sulphur-containing amino acids are in the region of 2×10^9 M^{-1} s^{-1}, in contrast with the four orders of magnitude lower k_q values for alanine (a non-sulphur-containing amino acid). Bearing in mind these results, we have examined the interaction of the lowest excited singlet state of Pyr^+ with sulphur-containing amino acids and carboxylic acids in aqueous solution.

The rate constants k_q of quenching of the Pyr^+ fluorescence by eight sulphur-containing amino acids, five sulphur-con-

Table 1
Rate constants for quenching of the Pyr$^+$ singlet state in aqueous solution at pH 7.0 (from lifetime measurements)

Number	Quencher	Formula	Z_AZ_B	$k_q \times 10^{-9}$ ($M^{-1} s^{-1}$)[a]	$k_q^{\mu=0} \times 10^{-9}$ ($M^{-1} s^{-1}$)
1	Methionine	$CH_3–S–CH_2–CH_2–CH(NH_3^+)–COO^-$	0	4.06 ± 0.10	–
2	α-Methylmethionine	$CH_3–S–CH_2–CH_2–C(CH_3)(NH_3^+)–COO^-$	0	4.15 ± 0.10	–
3	S-Methylcysteine	$CH_3–S–CH_2–CH(NH_3^+)–COO^-$	0	3.75 ± 0.10	–
4	S-Ethylcysteine	$CH_3–CH_2–S–CH_2–CH(NH_3^+)–COO^-$	0	4.14 ± 0.10	–
5	Ethionine	$CH_3–CH_2–S–CH_2–CH_2–CH(NH_3^+)–COO^-$	0	4.37 ± 0.14	–
6	Thiaproline	$\overline{HN-CH_2-S-CH_2-CH}-COO^-$	0	6.90 ± 0.12[b]	–
7	S-Carboxymethylcysteine	$^-OOC–CH_2–S–CH_2–CH(NH_3^+)–COO^-$	−1	13.10 ± 0.50[c]	16.0 ± 0.7
8	*N*-Acetylmethionine	$CH_3–S–CH_2–CH_2–CH(NHCOCH_3)–COO^-$	−1	12.80 ± 0.20[c]	15.6 ± 0.6
9	2-(Methylthio)ethanoic acid	$CH_3–S–CH_2–COO^-$	−1	12.70 ± 0.40[c]	15.5 ± 0.6
10	2,2′-Thiodiethanoic acid	$^-OOC–CH_2–S–CH_2–COO^-$	−2	16.10 ± 0.80[c]	23.9 ± 1.2
11	3,3′-Thiodipropionic acid	$^-OOC–CH_2–CH_2–S–CH_2–CH_2–COO^-$	−2	16.50 ± 1.00[c]	24.6 ± 1.4
12	3-(Carboxymethylthio)propionic acid	$^-OOC–CH_2–S–CH_2–CH_2–COO^-$	−2	16.90 ± 0.90[c]	25.2 ± 1.2
13	2-(Carboxymethylthio)succinic acid	$^-OOC–CH_2–S–CH(COO^-)–CH_2–COO^-$	−3	25.8 ± 1.70[c]	47.0 ± 2.1
14	Proline	$\overline{HN-CH_2-CH_2-CH_2-CH}-COO^-$	0	1.12 ± 0.13	–
15	Alanine	$CH_3–CH(NH_3^+)–COO^-$	0	1.00 ± 0.12	–
16	Glycine	$CH_2(NH_3^+)–COO^-$	0	0.92 ± 0.08	–

[a] Errors taken as twice the standard deviations from the least-squares fit.
[b] At pH 4.5 (99% of zwitterionic form), $k_q = (3.45 \pm 0.15) \times 10^9\ M^{-1}\ s^{-1}$.
[c] At constant ionic strength, $\mu = 0.03$ M.

taining carboxylic acids and three non-sulphur-containing amino acids were determined in aqueous solution at pH 7.0 at room temperature. Since only neutral solutions were studied, amino acids **1–5** and **14–16** are in their zwitterionic form [14,15], while compounds **7–9**, **10–12** and **13** carry net electric charges of −1, −2 and −3 respectively (Table 1). Thiaproline **6** under these experimental conditions exists as a mixture of ionic (approximately 70%) and zwitterionic (approximately 30%) forms (the pK_a value of thiaproline in aqueous solution at room temperature was determined to be p$K_a = 6.5 \pm 0.1$ [16]). The pure (99%) zwitterionic form of **6** can be observed at pH 4.5.

The lifetime of the Pyr$^+$ singlet state, in the absence of quencher, was measured to be $\tau_s^0 = 5.05 \pm 0.01$ ns (Fig. 1), which is in good agreement with the data presented in Ref. [12]. The fluorescence of Pyr$^+$ in the absence and presence of quencher can be adequately described by a single-exponential decay. The lifetime τ_s^0, fluorescence and absorption spectra are unaffected by a change in the ionic strength of the solution (in the range 0–0.5 M). The lifetime of the Pyr$^+$ singlet state decreases with the concentration of quencher used. The quenching rate constants k_q were obtained at constant ionic strength by employing the Stern–Volmer relation

$$\tau_s^0/\tau_s = 1 + k_q\tau_s^0[Q] \qquad (1)$$

where τ_s^0 and τ_s are the singlet state lifetimes in the absence and presence of quencher respectively.

Typical examples of the plots of τ_s^0/τ_s vs. [Q] are presented in Fig. 2.

The Stern–Volmer plots obtained from the lifetime measurements are perfectly linear over the whole range of quencher concentration used. There is no evidence of a transient effect [17], even for high concentrations of the quencher. Since both Pyr$^+$ and some of the quenchers exist in their ionic forms (in the experimental conditions used), we can expect [18] a dependence of the quenching rate constant on the square root of the ionic strength (μ) for compounds **7–13**. Such a typical dependence for various charges of the quencher molecules ($Z_A = +1$ and $Z_B = -1$, −2 or −3) is presented in Fig. 3.

The extrapolation of the k_q values measured for various ionic strengths to $\mu = 0$ permits a comparison of the quenching rate constants of the quencher molecules with different charges. In the case of quenching by amino acids in their zwitterionic form ($Z_B = 0$), the rate constant k_q is found to

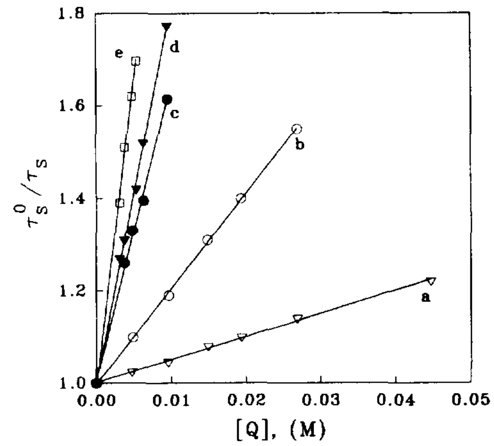


Fig. 2. Stern–Volmer plots for quenching of Pyr$^+$ fluorescence in aqueous solution at pH 7.0 by **15** (a), **1** (b), **8** (c), **10** (d) and **13** (e) (for compounds **8**, **10** and **13**, $\mu = 0.03$ M).

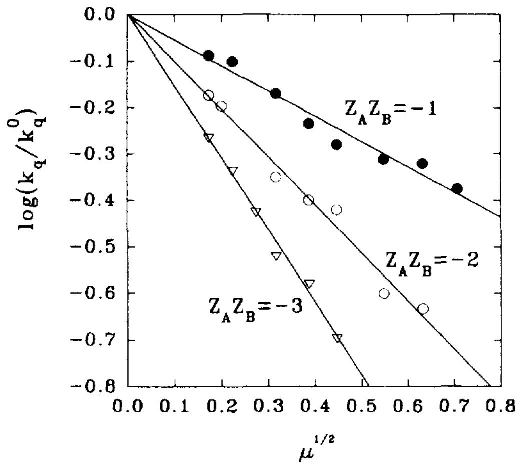


Fig. 3. Dependence of the quenching rate constants k_q on the square root of the ionic strength.

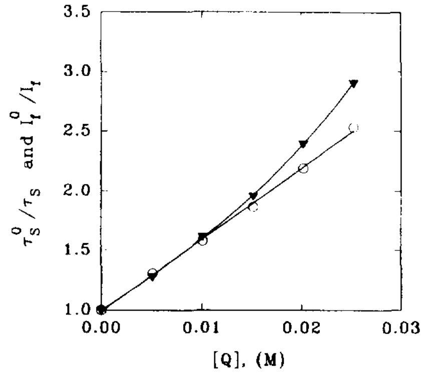


Fig. 4. Stern–Volmer plots for quenching of Pyr$^+$ fluorescence by 2,2'-thiodiethanoic acid ($Z_AZ_B=-2$) based on lifetime (circles) and fluorescence intensity (triangles) measurements.

be independent of the value of the ionic strength. The rate constants k_q for the quenching of the Pyr$^+$ fluorescence by sulphur-containing amino acids and carboxylic acids and non-sulphur-containing amino acids, determined for ionic strength $\mu=0.03$ M and extrapolated to $\mu=0$, are summarized in Table 1.

Contrary to the linear Stern–Volmer plots obtained from the lifetime measurements, the Stern–Volmer plots constructed from steady state measurements (Eq. (2)) are not linear over the whole range of quencher concentration used for compounds **7–13** carrying a negative charge ($Z_B=-1$, -2 or -3)

$$I_f^0/I_f=1+k_q\tau_s^0[Q] \qquad (2)$$

A typical plot of the fluorescence intensities I_f^0/I_f vs. [Q], showing a distinct upward curvature for high concentrations of quencher, is presented in Fig. 4. A similar effect was observed by Skalski et al. [12] for the quenching of Pyr$^+$ fluorescence by halide ions.

A comparison of the Stern–Volmer plots based on the fluorescence intensities and lifetime measurements indicates the participation of static and dynamic quenching [19] in the total quenching process. In spite of the inconsistency in the terminology of static quenching [20], a certain contribution of a ground state equilibrium between Pyr$^+$ and the quencher, leading to the formation of a non-fluorescent complex (ion pair association in the ground state), is responsible for the observed effect (Eq. (3))

$$\mathrm{Pyr}^+ + \mathrm{Q}^{-Z_B} \rightleftharpoons (\mathrm{Pyr}^+...\mathrm{Q}^{-Z_B}) \qquad (3)$$

However, the results of UV–visible absorption measurements indicate no changes in the absorption spectra due to ground state complex formation for the whole range of Pyr$^+$ and quencher concentrations used in the fluorescence experiments (and even for much higher concentrations: [Pyr$^+$] = 5×10^{-4} M and [Q] = 0.04 M). Thus the only argument for ground state complexation is based on the fluorescence intensity quenching experiments. To estimate the contribution of the static (K_S) and dynamic ($K_D=k_q\tau_s^0$) terms, the fluorescence quenching data were analysed by combining the intensity and lifetime quenching data according to [19]

$$I_f^0/I_f=(1+K_D[Q])(1+K_S[Q]) \qquad (4)$$

The results of such treatment are summarized in Table 2. A comparison of the K_D and K_S values indicates the main contribution of the dynamic term to the total quenching.

A lack of any change in the UV–visible absorption spectra due to ground state complex formation (this may be due to small values of the ion pair association constants K_S and relatively low concentrations of reactants due to their low solubility) does not allow any additional evidence to be presented, from an independent method, for ground state association between Pyr$^+$ and the sulphur-containing organic compounds in their ionic form. The results obtained indicate ion pairing in the ground state, but do not provide any basis for a conclusion concerning the possible structures of the suggested complexes.

Since the steady state fluorescence quenching data lead to a description of both static and dynamic quenching processes, we concentrate on the analysis of the results of time-resolved experiments (dynamic quenching) (Table 1). The rate constants k_q of quenching of the Pyr$^+$ fluorescence by sulphur-containing amino acids in their zwitterionic form (compounds **1–5**) are in the range $(3.7–4.4)\times10^9$ M^{-1} s^{-1}, approaching approximately one-half of the diffusion-controlled limit [10]. These values are twofold higher than the k_q values obtained for the quenching of the Pyr$^+$ triplet state [10] and CB triplet state [6] in aqueous solution. In the case

Table 2
Contribution of the dynamic and static terms in the quenching of Pyr$^+$ fluorescence by sulphur-containing organic compounds in aqueous solution

Compound	Z_AZ_B	μ (M)	K_D^a (M^{-1})	K_S^b (M^{-1})
8	−1	0.10	43.8 ± 2.6	5.2 ± 1.2
10	−2	0.20	59.9 ± 4.0	5.1 ± 1.0
13	−3	0.25	60.3 ± 1.6	11.7 ± 4.2

[a] From lifetime measurements (Eq. (2)).

[b] From Eq. (4), taking K_D from the lifetime measurements (errors taken as twice the standard deviations).

of thiaproline **6** at pH 7.0, k_q is determined to be 6.9×10^9 M^{-1} s^{-1}, which is higher than that of the amino acids **1–5**. As shown previously, in contrast with compounds **1–5**, thiaproline **6** exists in neutral solution as a mixture of ionic and zwitterionic forms. This leads to a higher value of k_q (due to the higher value of k_q for quenching of Pyr^+ by anions, see below) than expected. At pH 4.5, where 99% of thiaproline is in the zwitterionic form, the quenching rate constant is determined to be 3.45×10^9 M^{-1} s^{-1}, which is similar to the k_q values for **1–5** at pH 7.0 (Table 1).

On the other hand, for the amino acids alanine, proline and glycine, which lack a sulphur moiety, the k_q values are in the region of 1.0×10^9 M^{-1} s^{-1}, i.e. about four times lower than the k_q values obtained for the sulphur-containing amino acids. A similar effect (but with k_q values at least 3–4 orders of magnitude lower for the non-sulphur-containing amino acids) was also observed for the quenching of the Pyr^+ triplet state by sulphur-containing amino acids [11]. This may suggest that, as in the case of triplet state quenching, the quenching of the singlet state of Pyr^+ involves electron transfer from the sulphur atom (see below).

As expected for the reactions of cationic species with anions [18], the k_q values for quenching by sulphur-containing compounds in their anionic form increase in the sequence: $k_q(Q^0) < k_q(Q^{-1}) < k_q(Q^{-2}) < k_q(Q^{-3})$. In addition, for quenchers carrying the same charge, the k_q values are the same within experimental error (for compounds **1–6** ($Z_B = 0$), for **7–9** ($Z_B = -1$) and for **10–12** ($Z_B = -2$)). The average values of k_q for the quenching of the S_1 state of Pyr^+ by sulphur-containing organic compounds with various charges (Q^{-Z_B}) extrapolated to $\mu = 0$ are presented in the second column of Table 3. The effect of the reactant charge on the quenching rate constant can be explained by the change in the diffusion rate constant with the reactant charge $Z_A Z_B$. The diffusion rate constants k_d for the Pyr^+–Q^{-Z_B} systems can be estimated from Eq. (5) [18,21] if the diffusion coefficients D are known

$$k_d = 4 \times 10^{-3} \pi N_o (D_A + D_B) r_{HN} \qquad (5)$$

where $r_{HN} = r_c[\exp(r_c/r_{AB}) - 1]^{-1}$ and $r_c = Z_A Z_B e^2 / 4\pi\varepsilon\varepsilon_o kT$.

Table 3
Experimental quenching rate constants k_q and rate parameters for quenching of Pyr^+ fluorescence by sulphur-containing organic compounds in neutral aqueous solution at room temperature

$Z_A Z_B$	$k_q^a \times 10^{-10}$ ($M^{-1} s^{-1}$)	r_{AB} (Å)	$k_d^b \times 10^{-10}$ ($M^{-1} s^{-1}$)	$k_{-d}^c \times 10^{-10}$ (s^{-1})	$k_{el}^{o\,d} \times 10^{-10}$ (s^{-1})
0	0.41	7	1.0	1.2	0.77
−1	1.57	7	1.7	0.70	>1
−2	2.45	7	2.5	0.37	>1
−3	4.70	7	3.3	0.19	>1
		8	3.4	0.18	>1

[a] Average values for compounds **1–6**, **7–9** and **10–12** respectively (from Table 1).
[b] Calculated from Eqs. (5) and (6) taking $D_A + D_B = 2 \times 10^{-5}$ cm^2 s^{-1}.
[c] Calculated from Eq. (8).
[d] Estimated from Eq. (10).

In Eq. (5), N_o is Avogadro's number, r_{AB} is the distance required for the reaction between A* and B in the absence of ionic interaction (taken as the sum of the van der Waals' radii of A and B) and ε_o and ε are the dielectric constants of a vacuum and the solvent respectively. For uncharged reactants, the diffusion-limited rate constant is described by the Smoluchowski equation [18,21]

$$k_d = 4 \times 10^{-3} \pi N_o (D_A + D_B) r_{AB} \qquad (6)$$

The diffusion coefficients of Pyr^+ (D_A) and the various quenchers (D_B) in aqueous solution were estimated from the Stokes–Einstein equation with an empirical correction factor n [22]

$$D = kT / n\pi\eta r_w (f/f_o) \qquad (7)$$

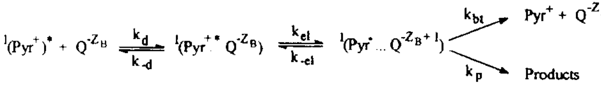
where η is the viscosity of the solvent, r_w is the van der Waals' radius of the diffusing species and f/f_o is the ratio of the frictional coefficient of the diffusing species to that of a spherical species. Eq. (7) was checked against two amino acids, alanine and glycine, whose diffusion coefficients in water are known [23]. Values of $D_A + D_B$, computed from Eq. (7), for Pyr^+ with each of the sulphur-containing quenchers do not change significantly (±6%). Also, the k_d values, calculated from Eqs. (5) or (6), are approximately constant (±3%) for series of equally charged quenchers, but increase with increasing negative charge of the quencher (Table 3). This suggests that the variation in k_d with $Z_A Z_B$ is responsible for the different rate constants k_q for various sulphur-containing organic compounds.

The quenching of the singlet state of Pyr^+ by sulphur-containing amino acids and carboxylic acids in neutral aqueous solution can be described by Scheme 1 [10], where k_{-d} is the dissociation rate constant of the encounter complex $^1(Pyr^{+*}...Q^{-Z_B})$, k_{bt} is the rate constant for back electron transfer, k_{el} and k_{-el} are the rate constants for electron transfer and k_p represents the rate constant of diffusion separation of the ion pair $^1(Pyr^{\cdot}...Q^{-Z_B}{}_{+1})$ and other reactions of the ion pair. The rate constant k_{-d} can be expressed by Eq. (8) [18,24]

$$k_{-d} = 3000 k_d [4\pi N_o (r_{AB})^3 \exp(-r_c/r_{AB})]^{-1} \qquad (8)$$

The involvement of electron transfer from the sulphur atom to the singlet state of Pyr^+ as the mechanism of quenching is supported by the following arguments:

1. the large values of the quenching rate constants (reaching the diffusion-controlled rate constants), which are similar to k_q for electron transfer quenching of Pyr^+ and CB triplets by sulphur-containing amino acids [5,6,10,11];
2. the k_q values for non-sulphur-containing amino acids **14–16** which are four times lower than those for quenchers containing the thioether moiety;



Scheme 1.

3. the large exoergonicity of the electron transfer process ($\Delta G_{\mathrm{el}} \approx -170$ kJ mol^{-1}, see below);
4. the analogy with the quenching of the Pyr$^+$ triplet state by sulphur-containing organic compounds shown to occur via electron transfer from the sulphur atom to the triplet state of Pyr$^+$ [11].

The standard free energy change for electron transfer from the sulphur atom to the S_1 state of Pyr$^+$ (the driving force for the process) was estimated to be $\Delta G_{\mathrm{el}} \approx -170$ kJ mol^{-1} from the Weller equation [25]: $\Delta G_{\mathrm{el}} = F(E_{\mathrm{ox}} - E_{\mathrm{red}}) - E_{\mathrm{S}} + \Delta w$. This value for ΔG_{el} was obtained by taking the oxidation potential for methionine as $E_{\mathrm{ox}} = 1.1$ V [10], the reduction potential of the Pyr$^+$ cation as $E_{\mathrm{red}} = -0.57$ V [11], the energy of the singlet state of Pyr$^+$ as $E_{\mathrm{S}} = 335$ kJ mol^{-1} [12] and $\Delta w = 0$ [11]. The large values of the quenching rate constants close to the diffusion-controlled limit can be explained in terms of a Rehm–Weller plot in electron transfer quenching [25], where a plateau region is observed for sufficiently exoergonic electron transfer processes. A similar diffusion-limited behaviour of $\log k_{\mathrm{q}}$ vs. ΔG_{el} has been observed for electron transfer quenching of substituted benzophenone triplets by methionine [10]. The lack of an experimentally observed Marcus inverted region for the highly exoergonic electron transfer processes in solution has been discussed since the appearance of the fundamental Rehm–Weller paper [25], and several possible explanations have recently been summarized in Ref. [26]. The large values for the quenching of Pyr$^+$ fluorescence by non-sulphur-containing amino acids **14–16** (k_{q} values of 1.0×10^9 M^{-1} s^{-1}) can be explained by a sufficiently high electron acceptor ability of the Pyr$^+$ excited singlet state ($-E_{\mathrm{red}} - E_{\mathrm{S}}$), which allows for electron transfer quenching from the –COO$^-$ group of the amino acids.

All the above-mentioned arguments, suggesting electron transfer from the sulphur atom to the singlet state of Pyr$^+$ as the mechanism of quenching, can be treated as indirect evidence for this process. An observation of electron transfer intermediates, i.e. Pyr$^{\cdot}$ radical and intermolecularly (S∴S)-bonded radical cation (in the flash photolysis experiments), would provide direct experimental evidence for the suggested process. In addition, for the reaction sensitized by the S_1 state, by analogy with the mechanism of photo-oxidation of sulphur-containing amino acids sensitized by the Pyr$^+$ triplet state [11], we would expect a disappearance of the reactants and the formation of CO_2 which could be measured by steady state photolysis. Both types of experiment (flash photolysis and steady state photolysis) should be performed for high concentrations of quenchers leading to substantial (more than 99%) quenching of the singlet state to avoid the contribution of reactions from a reactive Pyr$^+$ triplet state [11]. However, due to the limited solubility of sulphur-containing compounds in water and the short lifetime of the Pyr$^+$ singlet state ($\tau_{\mathrm{s}}^{0} = 5.0$ ns, Fig. 1), it was possible to quench, at the most, about 80% of the singlets (e.g. for [methionine] = 0.2 M). In these experimental conditions, the quantum yield of Pyr$^+$ disappearance was measured to be $\Phi_{\mathrm{Pyr}^+} \approx 0.01$ and CO_2 was not detected ($\Phi_{CO_2} < 0.02$). The appropriate values of the quantum yields for the reaction sensitized by the Pyr$^+$ triplet state were $\Phi_{\mathrm{Pyr}^+} = 0.20 \pm 0.03$ and $\Phi_{CO_2} \approx 0.08$ [11]. A comparison of the quantum yields in these two experiments indicates that the singlet state of Pyr$^+$ is not involved in the photochemical channel. The same conclusion can be drawn on the basis of nanosecond flash photolysis experiments, since Pyr$^{\cdot}$ radicals formed via a singlet quenching mechanism are not observed (low concentration of Pyr$^{\cdot}$ ($\Phi_{\mathrm{Pyr}^{\cdot}} < 0.03$) in the flash photolysis experiments was attributed to the reaction occurring from the Pyr$^+$ triplet state).

The results obtained suggest a fast back electron transfer reaction k_{bt} as the main reaction of the singlet radical ion pair $^1(\mathrm{Pyr}^{\cdot}...\mathrm{Q}^{-Z_{\mathrm{B}}}{}_{+1})$ (Scheme 1). The lack of photochemical reactions sensitized by Pyr$^+$ in the singlet state can be explained by an inefficient charge separation process of the singlet radical ion pair, which potentially could lead to Pyr$^{\cdot}$ radicals, the disappearance of reactants and the formation of CO_2. In the case of the reactions occurring from the Pyr$^+$ triplet state [11], the back electron transfer within the triplet radical ion pair $^3(\mathrm{Pyr}^{\cdot}...\mathrm{Q}^{-Z_{\mathrm{B}}}{}_{+1})$ is a spin forbidden process [27]. This, in principle, should allow for a concurrent charge separation channel and, as a consequence, for a photochemical reaction leading to the formation of the Pyr$^{\cdot}$ radical and sulphur-centred radical cation followed by decarboxylation (see scheme 1 in Ref. [11]).

Applying a steady state approximation to the intermediates in Scheme 1 leads to [10]

$$k_{\mathrm{q}} = k_{\mathrm{d}}\{1 + k_{-\mathrm{d}}/k_{\mathrm{el}} + k_{-\mathrm{d}}k_{-\mathrm{el}}/[(k_{\mathrm{p}} + k_{\mathrm{bt}})k_{\mathrm{el}}]\}^{-1} \qquad (9)$$

The limiting value of k_{q} for exoergonic electron transfer ($k_{\mathrm{el}} >> k_{-\mathrm{el}}$), corresponding to the plateau value on a Rehm–Weller plot, can be obtained from

$$k_{\mathrm{q}}^{\mathrm{max}} = k_{\mathrm{el}}^{\mathrm{o}}k_{\mathrm{d}}(k_{\mathrm{el}}^{\mathrm{o}} + k_{-\mathrm{d}})^{-1} \qquad (10)$$

where $k_{\mathrm{el}}^{\mathrm{o}}$ is the pre-exponential factor in the relation $k_{\mathrm{el}} = k_{\mathrm{el}}^{\mathrm{o}} \exp(-\Delta G_{\mathrm{el}}^{\ddagger}/RT)$. Thus the large values of k_{q} obtained for the quenching of the Pyr$^+$ fluorescence by $\mathrm{Q}^{-Z_{\mathrm{B}}}$ can be rationalized in terms of different values of the rate constants in Eq. (10). Since the values of k_{d} and $k_{-\mathrm{d}}$ can be estimated from Eqs. (5), (6) and (8) for known values of r_{AB} (taken as the sum of the van der Waals' radii of Pyr$^+$ and $\mathrm{Q}^{-Z_{\mathrm{B}}}$ [22]) and the $k_{\mathrm{q}}^{\mathrm{max}}$ values are the experimentally observed quenching rate constants (see Table 3), we can estimate $k_{\mathrm{el}}^{\mathrm{o}}$ for various quenchers based on Eq. (10). The results of such a treatment are summarized in Table 3 for different Pyr$^+$–$\mathrm{Q}^{-Z_{\mathrm{B}}}$ systems. For $Z_{\mathrm{A}}Z_{\mathrm{B}} = 0$, k_{q} was about half of the diffusion rate constant, and $k_{\mathrm{el}}^{\mathrm{o}}$ was estimated to be 0.77×10^{10} M^{-1} s^{-1}. In the case of quenchers in ionic form ($Z_{\mathrm{A}}Z_{\mathrm{B}} < 0$), the quenching was diffusion controlled ($k_{\mathrm{q}} \approx k_{\mathrm{d}}$) and the values of the rate constants for electron transfer $k_{\mathrm{el}}^{\mathrm{o}}$ were estimated to be much higher than 1×10^{10} M^{-1} s^{-1} (Table 3). Larger values of $k_{\mathrm{el}}^{\mathrm{o}}$ for quenchers carrying negative charges in comparison with compounds **1–6** ($Z_{\mathrm{A}}Z_{\mathrm{B}} = 0$) can be explained by the decrease in the effective distance between the ions in the $^1(\mathrm{Pyr}^{+*}\ldots\mathrm{Q}^{-Z_B})$ complex (Scheme 1) due to coulombic interaction (see Ref. [28]).

4. Conclusions

In summary, we have shown that the quenching of the Pyr^+ fluorescence by sulphur-containing amino acids and carboxylic acids (Q^{-Z_B}) in aqueous solution can be interpreted in terms of electron transfer from the sulphur atom to the Pyr^+ singlet state, followed by fast back electron transfer within the singlet radical ion pair $^1(\mathrm{Pyr}^{\cdot}\ldots\mathrm{Q}^{-Z_B}{}_{+1})$. The quenching was found to be a diffusion-controlled process depending on the reactant charge.

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