Fluorescence quenching of pyrene monomer and excimer by CH$_3$I

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Abstract

The fluorescence quenching rate constants of pyrene monomer and excimer by CH$_3$I were obtained at several temperatures in methylcyclohexane. Both quenching processes are kinetically controlled, allowing insight on the mechanism of quenching. The rate constants have both temperature-independent and temperature-dependent components. The temperature-independent component for both monomer and excimer fluorescence is consistent with quenching due to enhanced intersystem crossing to a lower energy triplet state. The monomer temperature-dependent component comes from the enhancement of the intersystem crossing to a higher energy triplet state. The thermally activated excimer quenching is associated with the excimer dissociation step to give a pyrene in a second triplet state plus a ground state pyrene molecule. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

The quenching of the fluorescence by heavy atoms was identified as physical quenching by Perrin in 1926 [1,2]. Afterwards, the theoretical and experimental results of McClure [3] and the detection by Kasha [4] of a yellow color due to the enhancement of the T$_1$ ← S$_0$ transition of α-chloronaphthalene when mixed with ethyl iodide, definitively demonstrated the heavy-atom effect.

Fluorescence quenching results from the enhancement of the intersystem crossing radiation-less transition and is effective when the heavy atom perturbs the spin–orbit coupling which requires the overlap of the orbitals of both the heavy atom and the fluorophore [5]. To be an effective quencher the heavy atom should be present in the fluorophore (internal heavy atom) or in a quencher molecule (external heavy atom) in proximity of the fluorophore [5,6].

Following the initial studies of McClure and Kasha, several quenching measurements were performed with aromatic molecules in liquid solution [7–14], in low temperature matrices [14–16], in doped molecular crystals [17–19], or forming rare-gas van der Waals clusters [20–22].

The external heavy-atom quenching is usually treated as a bimolecular process with rate constant dependent on the nature of the quencher. The
quenching occurs when the quencher is in the proximity of the fluorophore and two main mechanisms have been invoked to interpret experimental results: electron exchange [23–25] and charge transfer [11–13,26–29]. For inefficient quenchers and once the quenching occurs without the formation of a stable complex, the bimolecular rate constant is simply given by the increase of the intersystem-crossing rate constant divided by the molecular volume (M⁻¹) of the encounter complex [30]. In this case the quenching and the intersystem-crossing rate constants should be correlated. Indeed, Patterson and Rzad [31] observed that the quenching rate constant of several aromatic fluorophores by cesium chloride varies linearly with the fluorophore intersystem-crossing rate constant and Dreeskamp and Pabst [32] obtained a linear correlation between the log of the quenching rate constant of substituted anthracenes by iodopropane and the intersystem-crossing activation energy.

Following our previous results on the quenching of pyrene by alkyl iodides at room temperature [24], the quenching of pyrene by iodomethane (CH₃I) was studied at several temperatures. Iodomethane was chosen since the quenching is reaction limited and on the other hand pyrene and iodomethane do not form stable complexes. The contribution of the charge transfer process to the quenching is negligible since solvent polarity does not influence the quenching rate constant [24].

Using several concentrations of pyrene the quenching rate constants of the excited monomer and excimer were obtained. The quenching from both the excited monomer and excimer proceeds by both temperature-independent and temperature-dependent channel routes.

2. Kinetic scheme

The fluorescence spectrum of concentrated pyrene solutions is composed of a structured band in the blue associated with the monomer emission and a broad one at longer wavelengths characteristic of the excimer emission. Both monomer and excimer fluorescence intensities decrease in the presence of quenchers [24]. The kinetics can be described by Scheme 1, where $k_1$ describes the excimer (1D⁺) formation step occurring when an electronically excited monomer (1M⁺) encounters a ground state monomer (1M). Once formed the excimer can dissociate back with rate constant $k_{-1}$ to regenerate the excited plus ground state monomers. Both the excited monomer and excimer can still decay with intrinsic rate constants $k_M$ and $k_D$, or be quenched with rate constants $k_{QM}$ and $k_{QD}$, respectively.

After a δ-pulse of excitation light the monomer fluorescence intensity decays as a sum of two exponential terms,

$$I_M(t) = a_1 \exp(-\lambda_1 t) + a_2 \exp(-\lambda_2 t)$$  \hspace{1cm} (1)

while the excimer fluorescence intensity is described by a difference of two exponential terms,

$$I_D(t) = a_3[\exp(-\lambda_1 t) - \exp(-\lambda_2 t)]$$  \hspace{1cm} (2)

where

$$2\lambda_{1,2} = (X + Y) \mp \sqrt{(Y - X)^2 + 4k_1|M|k_{-1}}$$  \hspace{1cm} (3)

with

\begin{align*}
X &= k_M + k_1[M] + k_{QM}[Q], \\
Y &= k_D + k_{-1} + k_{QD}[Q]
\end{align*}  \hspace{1cm} (4)

and

\[\text{Scheme 1.}\]
\[
\frac{a_2}{a_1} = \frac{(X - \lambda_1)}{(\lambda_2 - X)}. \tag{5}
\]

The above equations are valid in the case of time-independent rate constants for the excimer formation and quenching rate constants. This is a reasonable approximation for low viscosity solvents \((k_1 \text{ can be considered constant in the ns time range})\) and for inefficient quenchers \((k_{QM} \text{ and } k_{QD} \text{ are not diffusion controlled})\). The monomer and excimer quenching rate constants can be obtained from the long decay constant, \(\lambda_1\). Indeed for low pyrene concentrations,

\[
\lambda_1 = k_M + k_1[M] + k_{QM}[Q] \tag{6}
\]

and for very high pyrene concentrations,

\[
\lambda_1 = k_D + k_{QD}[Q] \tag{7}
\]

while for all concentrations,

\[
\lambda_1 + \lambda_2 = k_M + k_D + k_1[M]
+ k_{-1} + (k_{QM} + k_{QD})[Q]. \tag{8}
\]

The slopes of the straight lines predicted by Eqs. (6) and (7) for the variation of \(\lambda_1\) with the concentration of quencher give directly \(k_{QM}\) and \(k_{QD}\), while from Eq. (8) the sum of the quenching rate constants are obtained.

3. Experimental

Pyrene from Fluka was zone refined. Iodomethane, (CH\(_3\)I) Aldrich-Gold Label, was used without further purification. Methylcyclohexane (Merk-Uvasol) was used as received. Solutions of pyrene in methylcyclohexane \((2 \times 10^{-6}, 1.0 \times 10^{-3} \text{ and } 5.2 \times 10^{-2} \text{ M})\) with the quencher \((0-0.4 \text{ M})\) were prepared and kept in the dark to avoid photodegradation. The solutions were degassed using the freeze-pump-thaw technique and sealed under a vacuum better than \(10^{-5} \text{ Torr}\). The UV–VIS absorption spectra were recorded in a Perkin–Elmer (model Lambda 15) spectrophotometer. Fluorescence spectra were obtained in a Spex Fluorolog 112 spectrofluorometer. Fluorescence decay curves were obtained by the single photon timing technique using picosecond laser excitation. The 327 nm excitation pulse (5–6 ps) was achieved by doubling the output light of a Coherent 701-2 dye (DCM) laser synchronously pumped by a mode-locked Coherent Innova 400-1 argon ion laser. Neutral density filters were used in the excitation pathway in order to avoid degradation of the samples. The monomer fluorescence \((\lambda = 376 \text{ nm})\) and the excimer fluorescence \((\lambda = 520 \text{ nm})\) were selected by a Jobin-Yvon HR320 monochromator with a grating of 100 lines mm\(^{-1}\) and detected by a Hamamatsu 2809U-01 microchannel plate photomultiplier. Two sets of experiments were performed for each sample and no significant differences were observed from one to the other set of decay measurements. This indicates that photodegradation during the experiments is unimportant. The temperature was controlled with a precision better than 0.5°C by means of an Oxford Instruments liquid nitrogen cryostat (DN 1704) for temperatures up to room temperature or by a home built thermostat for higher temperatures. Decay curves were analyzed using an iterative reconvolution method based on the algorithm of Marquardt [33,34].

4. Results and discussion

The UV–VIS absorption spectra of pyrene do not vary in the presence of the quencher, showing that no ground state complexes are formed between pyrene and CH\(_3\)I in the range of quencher concentrations used. Fig. 1 shows the fluorescence spectra of a \(1.0 \times 10^{-3} \text{ M pyrene solution in methylcyclohexane without quencher and with a concentration of 0.2 M of CH\(_3\)I. Both the pyrene monomer and excimer emissions decrease in the presence of the quencher. The shapes of the fluorescence spectra of a \(1.0 \times 10^{-3} \text{ M pyrene solution in the presence and in the absence of quencher are invariant with pyrene excitation at 368 nm (S\(_1\) – S\(_0\)) or at 345 nm (S\(_2\) – S\(_0\)) transition. This excludes the possibility of other species absorbing in the 360 nm wavelength regions where pyrene absorption is very low \((e \sim 100 \text{ M}^{-1}\text{cm}^{-1})\). On the other hand the excitation spectra of pyrene dilute solutions at several emission wavelengths covering the monomer and
excimer regions are identical and reproduce the UV absorption spectrum. Both the UV–VIS absorption and fluorescence measurements point to the absence of stable pyrene–pyrene and pyrene–CH₃I aggregates.

The decay curves of dilute pyrene solutions (2.0 × 10⁻⁶ M) in the absence and presence of CH₃I are single exponential with decay constant \( \lambda_1 \). This decay constant increases with the concentration of CH₃I, due to pyrene monomer fluorescence quenching. For concentrated pyrene solutions (1.0 × 10⁻⁵ and 5.2 × 10⁻² M), the excimer decay curves recovered at \( \lambda_{em} = 520 \) nm where monomer emission is negligible, grow with a fast rise time component \( (\tau_2 = 1/\lambda_2) \) and decay with a long lifetime \( (\tau_1 = 1/\lambda_1) \). The monomer decays as a sum of two exponentials with lifetimes similar to the ones recovered from the corresponding excimer decay curves. The global analysis of both decay curves allows the determination of common lifetimes for both decays and the ratio of the pre-exponential factors. The ratio of the pre-exponential factors for the excimer decay is close to -1.0 as predicted by Eq. (2), while the ratio for the monomer is dependent on both pyrene and quencher concentrations (Eq. (5)).

Fig. 2A and B show the plot of the long decay component, \( \lambda_1 \), versus the concentration of CH₃I at two temperatures for dilute (2 × 10⁻⁶ M) and concentrated (5.2 × 10⁻² M) pyrene solutions, respectively.

Both plots are linear according to Eqs. (6) and (7) and from the respective slopes the monomer, \( k_{QM} \), and the excimer, \( k_{QD} \), quenching rate constants were obtained. The intercepts give the intrinsic rate constants of the monomer \( (k_M) \) and excimer \( (k_D) \) for dilute and concentrated pyrene solutions, respectively. The sum of decay constants, \( \lambda_1 + \lambda_2 \), should also be linear with the concentration of CH₃I at each temperature as predicted by Eq. (8). Fig. 3A shows that this relation is indeed observed and from the slope the sum of the quenching rate constants, \( k_{QM} + k_{QD} \), were obtained.

These values, plotted in Fig. 3B for several temperatures, compare well with the sum of the quenching rate constants obtained from very dilute (2.0 × 10⁻⁶ M) and very concentrated (5.2 × 10⁻² M) pyrene solutions.

This confirms that kinetic Scheme 1 is obeyed and that the quenching rate constants are correctly obtained from the procedure described above.

Fig. 4A and B show the variation of \( k_{QM} \) and \( k_{QD} \) with the reciprocal of temperature.

The variation of both quenching rate constants with temperature can be fitted by the equation

\[
k_i = k_{i0} + A_i \exp(-E_i/RT)
\]

where the first term describes the non-activated processes, while the second considers the activated ones. The values for both monomer and excimer quenching rate constants of the non-activated processes \( (k_{QM0}, k_{QD0}) \) as well as the pre-exponential values \( (A_{QM}, A_{QD}) \) and the activation energies \( (E_{QM}, E_{QD}) \) for the activated ones are shown in Table 1.
The intrinsic rate constants for the deactivation of the monomer ($k_M$) and of the excimer ($k_D$) vary with temperature as shown in Fig. 5A, B.

The fit of $k_M$ and $k_D$ to Eq. (9) allows the determination of the rate constants for the non-activated processes ($k_{M0}, k_{D0}$) and of the pre-exponential factors ($A_M, A_D$) and activation energies ($E_M, E_D$) of the activated ones. The values of the fitted parameters are also included in Table 1.

The bimolecular quenching rate constants, $k_{QI}$ (M$^{-1}$ s$^{-1}$), are at least two orders of magnitude smaller than the rate constant for a bimolecular diffusion controlled process and can be related to the enhancement of the unimolecular spin-forbidden processes with rate constant, $\Delta k_{QI}$ (s$^{-1}$). The quenching should proceed by formation of a momentary encounter complex between the fluorophore (I) and the quencher Q, with rate constants $k_d$ and $k_{d-}$ for the formation and dissociation steps, respectively.

$$\Gamma + Q \rightarrow ^* (IQ)^* \rightarrow I + Q$$

and then

$$k_{QI} = \frac{\Delta k_{QI} k_d}{\Delta k_{QI} + k_{d-}} = \frac{\Delta k_{QI} K_{QI}}{1 + \frac{\Delta k_{QI}}{k_d}}.$$  \hspace{1cm} (10)

$K_{QI} = k_d/k_{d-}$ being the equilibrium constant for the formation of the encounter complex. For an inefficient quenching,

$$\frac{\Delta k_{QI}}{k_{d-}} \ll 1$$  \hspace{1cm} (11)

and then

$$k_{QI} = \Delta k_{QI} K_{QI}.$$  \hspace{1cm} (12)
A more general derivation of Eq. (12) that includes the lifetime of the fluorophore has been already described [30]. In the absence of strong electrostatic interactions between I and Q, the equilibrium constant is simply the molar volume $V_{QI}$ of the transient encounter complex, [30] and then

$$\Delta k_{QI} = \frac{k_{QI}}{V_{QI}}$$

where the encounter complex molar volume is given by

$$V_{QI} = \frac{4}{3}\pi(r_I + r_Q)^3N_A,$$

$r_I$ being the radius of the monomer or excimer, $r_Q$ the radius of the quencher and $N_A$ the Avogadro's number. The molar volumes for the monomer, $V_{QM} = 0.52$ M$^{-1}$, and excimer $V_{QD} = 0.83$ M$^{-1}$ were calculated knowing the radius of pyrene monomer, $r_M = 3.5$ A, excimer, $r_D = 4.5$ A and quencher, $r_Q = 2.4$ A [35]. The molar volumes are close to 1 M$^{-1}$, which implies that the magnitude of the bimolecular quenching rate constants and of the corresponding $\Delta k$ are similar.

In order to discuss the rate constant activation energies, the energy level diagram of pyrene monomer singlet and triplet states and the first excimer singlet state are shown in Fig. 6 [6,36].

The activation energy for the quenching process of the monomer ($E_{QM} = 2.2$ kcal mol$^{-1}$) is similar to that determined for the variation of $k_M$ ($E_M = 2.3$ kcal mol$^{-1}$). These values agree with the ones found by Stevens [37–39] and Jones [40]. As the heavy atom is not expected to perturb the singlet–singlet transitions (fluorescence and inter-
nal conversion) the activation energy is likely to be associated with the intersystem crossing to a higher triplet state. For pyrene, the activated intersystem crossing occurs to the second triplet state, T2, which is practically isoenergetic with the singlet state ($A_{T_2S_1} = 0.86 \text{ kcal mol}^{-1}$). The intersystem crossing to the third triplet state, T3, is negligible since the triplet–singlet energy gap is very high, $A_{T_3S_1} = 25.5 \text{ kcal mol}^{-1}$. However, the value of 2.2 kcal mol$^{-1}$ is higher than the singlet–triplet splitting, $A_{T_2S_1} = 0.86 \text{ kcal mol}^{-1}$, and can be explained by the possibility of accessing higher vibronic levels of $S_1$ strongly coupled to the triplet manifold. The association of the activation energy with the solvent viscous flow process was excluded by Stevens et al. [37], who obtained similar activation energies in both ethanol and paraffin oil. The rate constant for the non-activated process,
$k_{M0} = 1.7 \times 10^6 \text{ s}^{-1}$, is the sum of the rate constants for the radiative process plus the non-activated intersystem crossing to the low lying triplet state, $T_1$. The internal conversion in pyrene is likely to be negligible since the $S_1-S_0$ energy gap is very high ($\Delta E_{S1S0} \approx 77 \text{ kcal mol}^{-1}$) implying a very low Franck–Condon factor between the zero-vibrational level of $S_1$ and the isoenergetic vibrational level of the ground state. The radiative rate constant is considered temperature independent since, $k_t = k_{00} \times n^2$ where $k_{00}$ is solvent and temperature independent and the refractive index $n$ is a slowly varying function of $T$. Iodomethane can be considered effective in removing the spin prohibition, since the intersystem crossing rate constant $k_{MC}^{MC} \approx 10^5 \text{ s}^{-1}$ [41] and $\Delta k_{QM0} = 7.9 \times 10^7 \text{ s}^{-1}$. A similar conclusion can be extracted from the comparison of the values of the pre-exponential factors of the activated processes ($A_M = 2.7 \times 10^7 \text{ s}^{-1}$ and $\Delta A_{QM} = 4.8 \times 10^9 \text{ s}^{-1}$). The pre-exponential factors consider the intersystem crossing at infinite temperature, from a high vibrational level of $S_1$ to an isoenergetic vibrational level of $T_2$. The higher values for the activated processes are justified by most favorable Franck–Condon factors resulting from the lower singlet–triplet energy gap ($\Delta E_{T2S1} \approx 0.86 \text{ kcal mol}^{-1}$ and $\Delta E_{S1T1} \approx 28.6 \text{ kcal mol}^{-1}$).

In dilute pyrene solutions the sum of the monomer fluorescence quantum yield ($\Phi_M$), and of triplet state production ($\Phi_T$) is unity due to the negligible internal conversion process. At high concentrations when pyrene excimer is formed, Medinger and Wilkinson [42] showed that the sum of the quantum yields of monomer fluorescence ($\Phi_M$), excimer fluorescence ($\Phi_D$) and triplet state production ($\Phi_T$) is lower than unity. In order to interpret the results they assume that, besides the back reaction, the excimer decays also by the following processes.
where \( k_{\text{f}} \) is the radiative rate constant, \( k_{\text{i}} \) the internal conversion rate constant and \( k_{\text{t}} \) describes the dissociation of the excimer to give a pyrene in an excited triplet state plus a singlet ground state molecule. The reciprocal intrinsic lifetime of the excimer (see Scheme 1) is then given by the sum of the above processes:

\[
k_D = k_{\text{f}} + k_{\text{i}} + k_{\text{t}}.
\]

(i) \( ^1\text{D}^* \rightarrow 2^1\text{M} + h\nu_D \)

(ii) \( ^1\text{D}^* \rightarrow 2^1\text{M} \)

(iii) \( ^1\text{D}^* \rightarrow 3^1\text{M} + 1^1\text{M} \)

The process (iii) is spin forbidden and should be influenced by the external heavy-atom effect.

The rate constant of the non-activated pyrene excimer quenching, \( \Delta k_{\text{QD0}} = 2.3 \times 10^7 \text{ s}^{-1} \), is of the same order of magnitude of the corresponding one for the monomer quenching \( \Delta k_{\text{QM0}} = 7.9 \times 10^7 \text{ s}^{-1} \), and is associated with the intersystem crossing to the lower dissociative triplet state of the excimer. Both the pre-exponential factor and the activation energy for the activated quenching are very high. These values can be explained if the quenching proceeds by the enhancement of the excimer dissociation step (iii) according to

\[
^1\text{D}^* + \text{Q} \rightarrow 3^1\text{M} + 1^1\text{M} + \text{Q}
\]

Indeed, the activation energy is higher than the value reported for the pyrene excimer binding energy \( (9-10 \text{ kcal mol}^{-1}) \) [6,43] but very close to the energy gap \( (\Delta E_{S_i} = 10.3 \text{ kcal mol}^{-1}) \) between the singlet state of the excimer \( (S_{1D} = 67.5 \text{ kcal mol}^{-1}) \) and the second triplet state of pyrene \( (T_2 = 77.8 \text{ kcal mol}^{-1}) \). The value of the pre-exponential factor, \( 1.2 \times 10^{14} \text{ s}^{-1} \), is typical of a vibrational frequency and can be associated with the frequency of pyrene excimer dissociation mode. A similar value \( (5.2 \times 10^{14} \text{ s}^{-1}) \) was obtained for the pre-exponential factor of the Arrhenius plot of the excimer dissociation rate constant, \( k_{-1} \). These rate constants were calculated, at each
temperature, from the parameters of the global fit of the monomer and excimer decays of a 1.0 \times 10^{-3} \text{ M pyrene solution using Eqs. (3)-(5).}

The activation energy for the excimer quenching is different from the activation energy of $k_D$. This is not surprising since $k_D$ has a contribution from the internal conversion process that is expected to be only slightly influenced by the quencher. The excimer internal conversion occurs by the relative motion of the two pyrene moieties with activation energy similar to the viscous flow activation energy [44]. However, the value found, $E_D = 3.8 \text{ kcal mol}^{-1}$, is higher than the viscous flow activation energy, $E_q = 2.5 \text{ kcal mol}^{-1}$, which was also observed in other solvents [45]. This could be explained by a small contribution to $k_D$ of the dissociation process (iii) whose activation energy is higher than the viscous flow activation energy. The fit of $k_D$ with a sum of two activation terms is also possible if the activation energies are fixed to $E_q$ and $E_{QD}$, confirming our reasoning and the complexity of the variation of $k_D$ with temperature.

5. Conclusions

The quenching rate constants for pyrene monomer and excimer by CH$_3$I in methylcyclohexane at several temperatures can be described as a sum of non-activated and activated processes. The CH$_3$I is effective in promoting the increase of the intersystem-crossing rate by a factor of $\sim 10^3$. In both pyrene monomer and excimer the non-activated processes result from the increase of the intersystem-crossing rate constant from the excited singlet state to a lower triplet state. The activated processes in pyrene monomer result from the enhancement of the intersystem crossing to the $T_2$ triplet state. The quenching activated process of the excimer results from the enhancement of the excimer dissociation step to give a pyrene molecule in a triplet state plus a ground state molecule. This spin-forbidden process competes with the spin-allowed back excimer dissociation reaction. Both excimer dissociation steps have high pre-exponential factors that are associated with the frequency of the excimer dissociation mode.

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