

Excimer Fluorescence Quenching of Poly (styrene-co-acrylic acid)-Eu Complex by Simple Hydrocarbons in Tetrahydrofuran Solutions

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Quenching of excimer fluorescence from polystyrene-acrylic acid copolymers containing Eu^{3+} has been studied in tetrahydrofuran solution using simple aromatic hydrocarbons as quenchers under steady-state conditions. Aromatic hydrocarbons quenched collisionally the excimer fluorescence and their rate constants of quenching were determined. The magnitude of quenching constant is interpreted in terms of the cube root of the molar volume of quencher. Cycloalkanes were not effective in quenching the excimer fluorescence possibly due to different solubility characteristics from aromatic hydrocarbons.

Introduction

Fluorescence quenching has been widely studied both as a fundamental phenomenon, and in the numerous applications of fluorescence to biochemical processes.¹ Since the quenching requires in principle molecular contact between fluorophore and quencher, quenching studies can reveal the accessibility of fluorophores to quencher, the diffusion rates of quenchers, and the localization of fluorophores in proteins and membranes.

Almost all studies of fluorescence quenching of excimer forming polymers such as aryl vinyl polymers have been made in fluid solutions, because of experimental simplicity. In rigid matrices, however, there are technical problems with adding sufficient quencher without eliminating the rigidity of such systems. The quenching of excimer fluorescence from polystyrene and methyl methacrylate copolymer with styrene has been studied to observe any occurrence of singlet energy migration in dilute solutions.² Fluorescence quenching of poly (2-vinylnaphthalene) and poly (N-vinylcarbazole) has been examined to estimate the singlet energy migration rate.³ Through fluorescence quenching for polystyrene and poly (*p*-methylstyrene) in solution the mechanism of excimer formation and the activation energy of excimer formation have been studied.⁴ In dilute polymer solution, excimer emission is known to proceed by energy migration through a polymer chain to an excimer trap on the same chain although no clear conclusion has been reached concerning the nature of energy migration processes. This is of particular interest due to the parallel between this process and that of photosynthesis.

While static quenching also occurs in fluid solutions, predominant mechanism is the diffusional encounter of an excited chromophore and a quencher molecule. As part of a continuing study on polystyrene-acrylic acid copolymers containing Eu^{3+} as a metal containing polymer the present paper is concerned with excimer fluorescence quenching by some simple aromatic hydrocarbons and a few cyclohexanes at room temperature and to find out the effect of quencher structure on the collisional quenching constant. The sequence of quenching constants was discussed with reference to the cube root of molar volume of quenchers.

Experimental

Reagents

Styrene-acrylic acid copolymer (PSAA) and PSAA-Eu complex were prepared as described previously.⁵ Spectroscopic grade of tetrahydrofuran (THF), benzene and toluene

were purchased from Tedia, Merck and Matheson-Coleman, respectively. Reagents grade of *o*-, *m*-xylene (from Junsei), ethylbenzene (from Ajax) and mesitylene (from Chameleon) were used. Spectroanalytical grade of cyclohexane (from Reidel) and ethylcyclohexane (from Shimakyu) and reagent grade of methylcyclohexane (from shimakyu) were tested as possible quenchers for excimer fluorescence.

Measurements

A Hitachi model 650-60 spectrofluorimeter was used to measure fluorescence spectra of polymers in THF. The concentration of polymer solutions was 4 mmol/l (chromophore unit) for most of the quenching experiments. The effects of quenchers upon the fluorescence intensity were assessed by measurements at the wavelength of maximum emission. The effect of temperature on the fluorescence quenching of excimer was measured to identify the nature of quenching. Temperature regulation was accomplished by circulating a temperature-controlled water in the cell holder. The emission of excimer was measured at 330nm having corrected from the contribution from monomer and quencher at this wavelength. Absorption spectra were recorded on a Cary 17D spectrophotometer to observe any ground state association. A Ubbelohde viscometer and a pycnometer were used to measure solution viscosity.

Results and Discussion

Figure 1 shows a typical variation in the fluorescence emission spectra of 4 mM PASS-Eu (3.0 wt%) complex in THF media excited at 260 nm as a function of toluene concentration. The broad band occurring near 330 nm arises from excimer fluorescence. Two additional spectral regions may be identified corresponding from styrene monomer (peak at around 294 nm) and added toluene at about 286 nm of which the latter overlaps with the emission due to Raman scattering from THF at 283 nm.

The decrease in excimer fluorescence intensity is small but clearly discernible by the addition of toluene. The styrene monomer band appears on a sloping background. Since the monomer band overlaps with neighboring bands of excimer emission and toluene fluorescence, the intensity of monomer, I_m , obtained from the sloping background contains comparatively large experimental errors. However, a careful examination revealed that I_m remained fairly constant over toluene concentration studied here as displayed in Figure 2. A similar observation was made here and previously by the use of benzene.⁵ Although the complete understanding on the variation of monomer fluorescence intensity will still require

a detailed study, we are inclined to believe that the decrease in excimer fluorescence intensity does not seem to result in the simultaneous increase in monomer fluorescence intensity. The dissociation of excimer may not contribute to the observed monomer emission. Also in the figure are included the intensities of excimer emission, I_e , and of toluene in the presence of 4 mM PASS-Eu complex, which were corrected for overlapping bands. In addition the fluorescence intensity of toluene alone in THF excited at 260 nm is compared.

In dilute solutions of PSAA-Eu complex in THF the excimer formation is almost entirely governed by intrachain interaction between phenyl groups on the same polymer chain. This is shown by the fact illustrated in Figure 3 that the ratio of excimer to monomer fluorescence intensities is nearly constant of polymer concentration for the complexes used here even though the ratio contains relatively large experimental uncertainties due to the difficulty to extract monomer intensity. At lower concentrations below 10^{-3} M, the ratios deviate

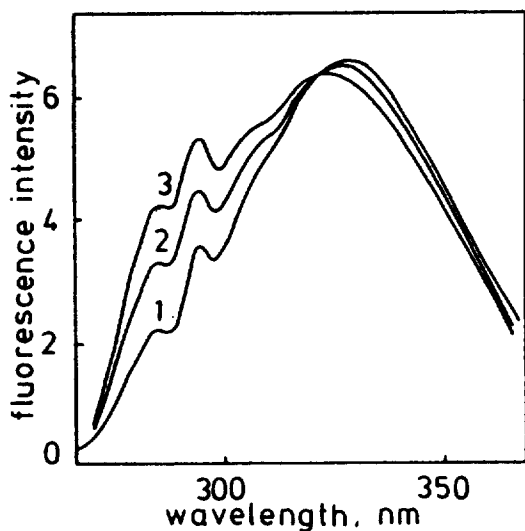


Figure 1. Fluorescence emission spectra of PSAA-Eu (3.0 wt%) complex in THF depending on toluene concentration (1) 0M; (2) 5×10^{-3} M; (3) 1×10^{-2} M. $\lambda_{ex} = 260$ nm.

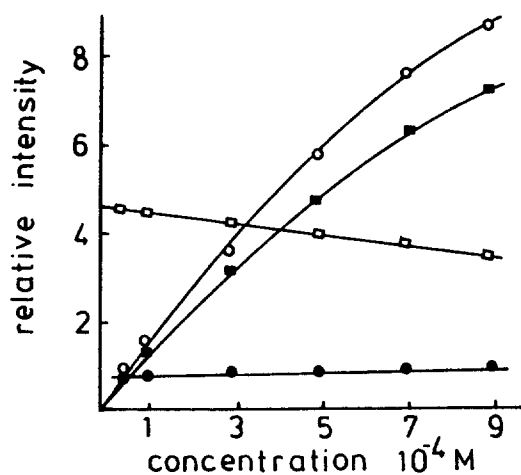


Figure 2. Relative fluorescence intensities of toluene at 286 nm with (■) and without (○) 4 mM PSAA-Eu (3.0 wt%) in THF, of excimer at 330 nm (□), and of styrene monomer at 294 nm (●) versus toluene concentration. $\lambda_{ex} = 260$ nm.

from constancy because of the errors caused by lower intensities of monomer and excimer. In order to exclude intermolecular effects, measurements were performed on dilute solutions of PSAA-Eu complex hereafter. At higher concentrations, intermolecularly formed excimers will contribute to the observed excimer fluorescence to result in an increase in the ratio, I_e/I_m .

We find that the excimer fluorescence is quenched by toluene and *vice versa*. The quenching of excimer fluorescence by aromatic hydrocarbons was analyzed according to the Stern-Volmer equation⁴

$$F_0/F = 1 + K_m[Q] = 1 + k_q\tau_0[Q]$$

where F_0 and F are the intensities of excimer fluorescence in the absence and presence of quencher, respectively; K_m , a constant given by the slope of the line; k_q , the second order rate constant for deactivation of excimer; τ_0 , the lifetime of excimer in the absence of quencher; and $[Q]$ the concentration of quencher. The variations of F_0/F with the concentration of quencher fell to straight lines (Figure 4) over the concentration range studied here for all the aromatic

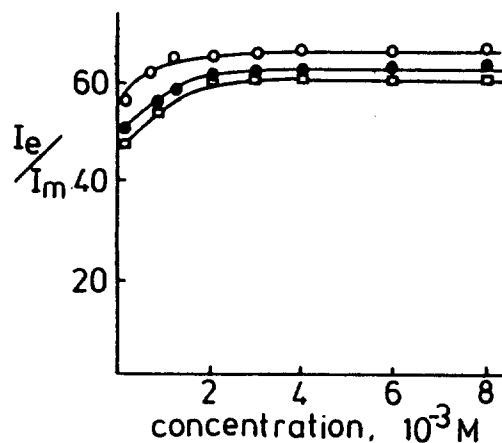


Figure 3. I_e/I_m versus concentration of PSAA (○), PSAA-Eu (1.0 wt%) (□) and PSAA-Eu (3.0 wt%) (●) in THF.

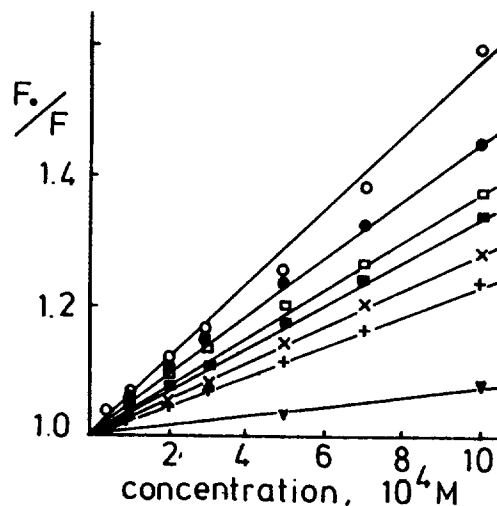


Figure 4. Stern-Volmer plots for the excimer fluorescence quenching by ethylbenzene, mesitylene, *o*-xylene, toluene, *p*-xylene, *m*-xylene and benzene from top to bottom, respectively, in 4 mM PSAA-Eu (3.0 wt%) THF solutions at 25°C.

hydrocarbons, which indicates a single class of excimers all equally accessible to quenchers.

To find the nature of excimer quenching by the benzene derivatives, quenching experiment was conducted at two different temperatures, 23° and 36°C. A representative result is displayed in Figure 5 for ethylbenzene as a quencher in THF solutions of 4 mM PSAA-Eu complex. An increase in temperature from 23° to 36°C results in approximately 15% increase in the slope and about 11% increase in the excimer fluorescence intensity for all concentrations of ethylbenzene without any absorption spectral change. The increase in F_0/F provides an evidence for the diffusive nature of the excimer fluorescence quenching. With the rise in temperature over the range studied the excimer fluorescence intensities in poly (1-naphthyl methacrylate),⁶ poly (2-vinyl naphthalene),⁷ 2,4-Di (2-naphthyl) pentane,⁸ and polystyrene and poly (*P*-methylstyrene)⁴ showed a slight decrease, but no change or slight increase below approximately 30°C. The apparent opposite behavior of the excimer fluorescence intensity with temperature in the present study indicates presumably that acrylic acid and europium restricts in the conformational change in PSAA-Eu complex and thus a larger activation energy of the excimer formation is required.

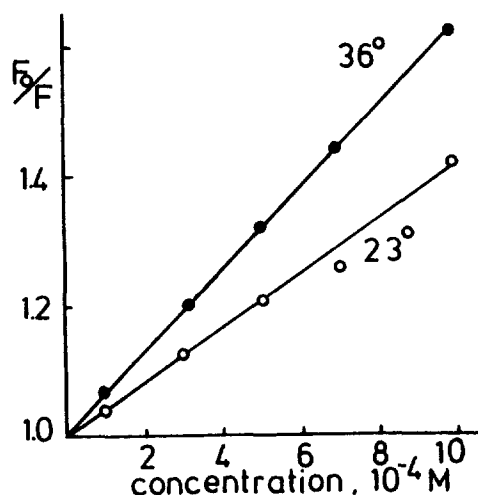


Figure 5. Stern-Volmer plots for the excimer fluorescence quenching by ethylbenzene in 4 mM PSAA-Eu (3.0 wt%) at 23°C and 36°C.

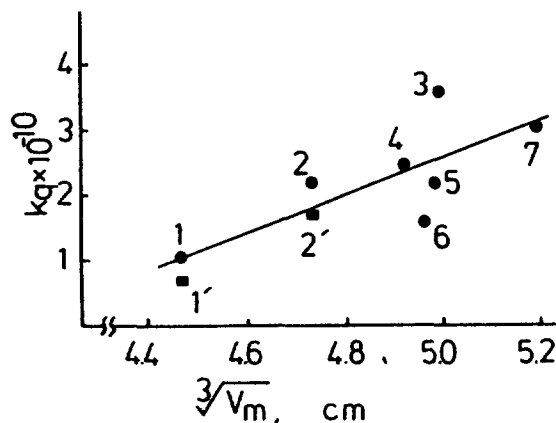


Figure 6. Plot of k_q versus $\sqrt[3]{V_m}$: (1) benzene; (2) toluene; (3) ethylbenzene; (4) *o*-xylene; (5) *p*-xylene; (6) *m*-xylene; (7) mesitylene. (1)' benzene and (2)' toluene are from reference 5.

The excimer lifetime, 15 nsec, for polystyrene-methylmethacrylate⁹ and polystyrene-0.01% 2-phenyl-(5-vinyl) phenyloxalate¹⁰ was assumed for that of PSAA-Eu complex to calculate k_q as a first approximation because the decay mechanism of excimer seems to be insensitive to the polymer composition when styrene is a predominant comonomer. The values of K_{sc} and k_q so determined from Figure 4 are listed in Table 1. The magnitudes of k_q lie in the range of diffusion-controlled quenching as estimated⁵ roughly with Smoluchowski equation, $k = 4\pi RDN/1000$, where R is generally the sum of the molecular radii of fluorescer and quencher, D is the sum of diffusion coefficients of fluorescer and quencher, and N is Avogadro number. The observed k_q values are somewhat larger than the estimated possibly because the encounter for fluorescence quenching could occur at a distance of approximately 10 Å in configurationally non-specific fashion even though the excimer is a short distance event at a distance of 3–5 Å¹¹.

The experimental facts the excimer fluorescence quenching is diffusive in nature and that no absorption spectral change was observed suggest it being difficult to assume that the benzene derivatives are associated with the phenyl group on polymer chain in the ground state. The dispersive force between phenyl groups on polymer chain and aromatic quencher molecules are considered to be small.

A relatively large variation of k_q between $3.6 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ for ethylbenzene and $1.0 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ for benzene was considered to result from either viscosity difference of the medium in the presence of quencher or size of the quencher molecule both of which may affect diffusion coefficients. The relative viscosities at 20°C of 4 mM PSAA-Eu complex in THF in the presence of 1 mM quencher with respect to the complex solution in the absence of quencher were obtained and are summarized in Table 1. As expected, the relative viscosity differences of less than 1.0% at most were negligible to cause any significant change of the quenching constant.

In the previous paper⁵ we tentatively stated that a bulkier quencher appeared to be more efficient in quenching the excimer fluorescence. To relate k_q with bulkiness the molecular size of quencher was estimated from the cube root of molecular volume, $\sqrt[3]{V_m}$, where V_m was obtained from molar mass divided by density¹² of the quencher. The $\sqrt[3]{V_m}$ values are

Table 1. Values of $\sqrt[3]{V_m}$, Quenching Constant and Relative Viscosity at 25°C of 4 mM PSAA in THF

Quencher	$\sqrt[3]{V_m}, \text{ cm}^3$	$k_q, \cdot 10^{10} \text{ M}^{-1} \text{ s}^{-1}$	η rel ^c
none	—	—	1.0000
benzene	4.472	1.0	1.008
toluene	4.745	2.3	1.0025
ethylbenzene	4.974	3.6	1.0029
<i>o</i> -xylene	4.949	2.5	1.0034
<i>m</i> -xylene	4.980	1.7	1.0041
<i>p</i> -xylene	4.986	1.9	1.0020
mesitylene	5.187	3.0	1.0120
cyclohexane	4.773	≤ 1.0	—
methylcyclohexane	5.044	≤ 1.0	—
ethylcyclohexane	5.231	≤ 1.0	—

^aMolar volumes (V_m) of quencher were calculated from the data given in reference 11; ^b $\tau_e = 15$ nsec from references 9 and 10; ^cQuencher concentration was 1.0 mM.

listed in Table 1 and a plot of k_q versus $\sqrt[3]{V_m}$ is displayed in Figure 6. The k_q values of benzene and toluene from the previous study⁵ are included. Although the data are scattered due to uncertainties in obtaining excimer fluorescence intensities there appears to exist a fair correlation between k_q and $\sqrt[3]{V_m}$. It seems not unreasonable to conclude that the diffusive quenching of the excimer fluorescence of PSAA-Eu complex by simple aromatic hydrocarbons depends on the molecular size of quencher, *i.e.*, the bulkier is the quencher, the more efficient is the quenching of excimer fluorescence.

A quite analogous experiments were performed with cyclohexane, methylcyclohexane and ethylcyclohexane to test that the size criteria can be applied to these cycloalkanes as well. Unlike benzene derivatives, however, the cycloalkanes essentially did not quench the excimer fluorescence of PSAA-Eu complex in THF. This result may not be related to the molecular size of cycloalkanes since their molar volumes were not much different from those of aromatic hydrocarbons as can be seen in Table 1. The different quenching behavior may be interpreted in terms of different solubilities of cycloalkanes with respect to styrene. Those benzene derivatives studied here have nearly the same solubility parameters as styrene but cycloalkanes have less.¹³ The phenyl groups on polymer chain apparently from unfavorable environment for cycloalkanes to approach close to the excimer forming sites.

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Separation of D and L Amino Acids by High-Performance Liquid Chromatography

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Separation of optical isomers of some derivatives of amino acids by reversed-phase HPLC has been accomplished by adding a chelate of an optically active amino acid to copper(II) to the mobile phase. Cu(II) complexes of L-proline and L-hydroxyproline in the mobile phase showed different degrees of separation. Optical isomers of DNS derivatives of amino acids are selectively separated, but those of several other derivatives are not at all. The kinds of buffer agents, the pH, and the concentrations of acetonitrile and the Cu(II) ligand all affect the separations. The elution behavior between D and L DNS-amino acids appears to depend on the alkyl side chain of the amino acids. A chromatographic mechanism is proposed that is based on a stereospecificity of the formation of ternary complexes by the D, L-DNS-amino acids and the chiral additive associated with the stationary phase. The steric effects of the ligand exchange reactions are related with the feasibility of cis and/or trans attack of the amino acids to the binary chiral chelate retained on the stationary phase.

Introduction

The resolution of optical isomers by a high performance liquid chromatography (HPLC) has been of great interest, especially for the separation of D, L-amino acids. The two general methods for the liquid chromatographic resolution of the optical isomers of amino acids have been used. One was the use of chiral ligand immobilized on the solid support to create an asymmetrical environment.¹⁻⁶ The other was the use of the addition of the chiral metal chelate to the mobile phase.⁷⁻¹⁹

Separation of the isomers on stationary phases bonded to a chiral ligand is particularly applicable to the isolation of racemates when recovery of the purified isomers is desired. Some of the phases have a limited applicability to only a few amino acid pairs. The required phases are seldom commercially available because of the complexity of their syntheses and the difficulties of separation of the optically active ligand from the synthetic mixture. These disadvantages have made the use of the chiral chelate addition a more attractive alternative. Karger *et al.*^{6,9} first reported the use of L-2-alkyl-4-octyldiethylenetriamine metal complexes in