Quenching of Fluorescence of Pyrene and Anthracene by Electron-accepting Substances

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In despite of many investigations,[1] the inner mechanism of fluorescence quenching is not clearly understood. It has been pointed out that fluorescence quenching by foreign substances might be closely related to the electron transfer reaction or the charge-transfer interaction between an excited fluorescent molecule and a quencher molecule.[2-8] If such a reaction or an interaction plays an important role in the mechanism of fluorescence quenching, the quenching ability of a quencher molecule may be connected with its electron-affinity or ionization potential.[9] As far as we know, however, only a few works[9-11] have been done along this line.

In this investigation, the electron-accepting substances were employed for quenching the fluorescence of pyrene and anthracene, and a study was attempted on the relation between the quenching constants and the electron-affinities of the quencher molecules, on the basis of the quenching mechanism involving the charge-transfer interaction between the fluorescent hydrocarbons and the quenchers.

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Experimental

Pyrene and anthracene were purified by recrystallization, zone-refining and sublimation in vacuum. The substances employed as quenchers were repeatedly recrystallized and then sublimed in vacuum. The solvents used were dried and distilled benzene for the pyrene solution and G. R. grade acetone for the anthracene solution.

The measurements of the fluorescence spectra and the fluorescence intensities were made with a Hitachi EPS-2 U recording spectrophotometer mounted with an attachment for fluorescence measurement. The light source for fluorescence excitation was a super-high pressure mercury lamp with a suitable UV filter (mainly $365\,m\mu$). The measurements were made at room temperature for the benzene solution of pyrene and the acetone solution of anthracene, the concentrations of which were 2.52×10^{-3} and 1.00×10^{-3} (mol/l), respectively. These solutions were not degassed, but it is usually assumed that the quenching action of a quencher is independent of dissolved oxygen.[8b]

Since the shape of the fluorescence spectra of these aromatic hydrocarbons did not practically change in the presence of a quencher, the relative fluorescence intensities were obtained from the heights at a given wavelength for the various quencher concentrations. At higher quencher concentrations, however, the absorption of exciting light by the quencher as well as the reabsorption of fluorescence gave rise to serious errors in the observed fluorescence intensities. They were appropriately corrected by taking account of the optical geometry of excitation and observation and of the quencher concetrations.[12]

From a plot of the relative fluorescence intensities against the logarithms of quencher concentrations, the quenching constant was obtained as the reciprocal of the half-value-concentration, where the value of the relative fluorescence intensity was reduced to half the value in the absence of the quencher.

12) The observed fluorescence intensities, $I_f{}^{obs}$, were corrected by the following formula:

$$I_f{}^{cor} = \frac{D_Q + D_A}{1 - 10^{-(D_Q + D_A)}} \frac{1 - 10^{-D_A}}{D_A} \frac{1 - 10^{-D_F}}{2.303\,D_F} I_f{}^{obs}$$
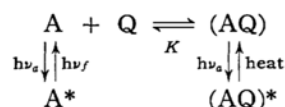
where D_A and D_Q are the absorbances at the exciting wavelength of a fluorescer and a quencher, respectively, and D_F is the absorbance of the solution at the wavelength where the fluorescence intensities were measured.

For the pyrene solution, the quenching constants were estimated for both monomer and excimer fluorescence bands with the peaks at 396 mμ and 480 mμ, respectively.[13]

Results and Discussion

The quenching constants obtained for a series of electron-accepting substances are shown in Table 1 together with the values of their electron affinities. For the pyrene solution the equilibrium constants, K, for charge transfer complex formation with these electron-accepting substances, are also given.

In studies on fluorescence quenching, it is important to know the type of the molecular process and the nature of the inner mechanism of fluorescence quenching. Two types of quenching processes, static and dynamical ones, can be assumed.[1,14] In a static quenching process, the non-fluorescent molecular complex, (AQ), is formed between a fluorescer, A, and a quencher, Q, in the ground state and only the uncomplexed fraction of the fluorescer contributes to the observed emission. This is given by the following scheme:

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$$A + Q \rightleftharpoons (AQ)$$

$$\begin{array}{ccc} & K & \\ h\nu_a \downarrow \uparrow h\nu_f & & h\nu_a \downarrow \uparrow \text{heat} \\ A^* & & (AQ)^* \end{array}$$

The equilibrium constant, K, is expressed as

$$K = \frac{[(AQ)]}{[A][Q]}.$$

If a static quenching process can be assumed, the quenching constant, k, may be approximately given by the following form:

$$k = \frac{K}{KC+1}$$

where C is the initial concentration of fluorescer. This equation can easily be derived for 1 : 1 complex formation, if the complexed fraction is sufficiently small, as is usually the case. For the pyrene solutions, as is seen from Table 1, $C = 2.52 \times 10^{-3}$ (mol/l) and $K = 0.1$—20 (l/mol); therefore, k should be 0.1—20, which is, however, inconsistent with the observed k-values, i.e., 10^2—10^3. The fact that the observed k-values are much larger than those estimated on the assumption of a static quenching process, leads to the predominance of the dynamical quenching process.[10,11,13,14] in which a quencher molecule encounters an excited fluorescer molecule by diffusion through the solution, forming an excited complex in which deactiva-

TABLE 1. QUENCHING CONSTANTS OF ELECTRON-ACCEPTING QUENCHERS FOR FLUORESCENCE OF PYRENE AND ANTHRACENE

Quencher	Electron-affinity[a] (eV)	K[b] (l/mol)	Quenching constant, k(l/mol)		Anthracene
			Pyrene		
			Monomer	Excimer	
Phthalic anhydride	0.15	c)	238	385	122
Maleic anhydride	0.57	c)	345	770	35[e]
Tetrachlorophthalic anhydride	0.58	2.7	303	500	227
p-Benzoquinone	0.6	c)	417	833	185
sym-Trinitrobenzene	0.7	3.7	476	909	278
Pyromellitic dianhydride	0.85	—	—	—	132
2,5-Dichlorobenzoquinone	1.15	c)	870	1110	370
p-Chloranil	1.37	c)	—	—	1100[d]
p-Bromanil	1.4	c)	2040[d]	2940[d]	—
2,3-Dicyanobenzoquinone	1.7	0.2	1790	2270	556
Tetracyanoethylene	1.8	19	2380	2380	376[e]
Iodine	(1.8)	c)	690	1000	—
2,4-Dinitrochlorobenzene	—	—	—	—	278
2,3-Dichloronaphthoquinone	—	—	—	—	1000[d]

a) G. Briegleb, Angew. Chem., 76, 326 (1964).
b) Estimated by the Benesi-Hildebrand's method.
c) The charge transfer spectra were not separably observed.
d) This large value may have resulted from the insufficient correction for the reabsorption of fluorescence by the quencher.
e) The observed small value is probably due to the Diels-Alder reaction.

tion of energy is effective. That the absorption spectra of the aromatic hydrocarbon were not affected by the addition of a quencher, is also favorable to a dynamical quenching process.

As for the inner mechanism, the possibility of the electronic energy transfer can be eliminated because of the small overlap of the fluorescence spectra of the fluorescers with the absorption spectra of the quenchers. Further, it can be seen from Table 1 that the quenching abilities of these quenchers can not consistently be explained by the heavy atom effect, which enhances the intersystem-crossing from the excited singlet state to the triplet state in the fluorescer molecule.

Comparing the k-values with the electron-affinities enables one to find a fairly good correlation between them. This parallelism between the quenching constants[15] and the electron-affinities suggests that the charge transfer interaction should play an important role in the fluorescence quenching mechanism for these systems.

The charge transfer mechanism of fluorescence quenching may be interpreted in terms of the formation of excited-state charge transfer complex due to the increased mixing of the locally excited (A*Q) and the charge transfer (A$^+$Q$^-$) configurations on the mutual approach of A* and Q.[6,7]

For the systems of pyrene-phthalic anhydride and pyrene-tetrachlorophthalic anhydride, the complex emissions were observed at $510\,\mathrm{m}\mu$

and $550\,\mathrm{m}\mu$,[16] respectively, in benzene solution at room temperature. The intensity of these complex emissions increased with the rise of temperature. Since the viscosity of the solvent decreases at higher temperatures, the molecules diffuse in solution more easily to form the excited complexes, resulting in the complex emission. This phenomenon will also support a diffusional process in excited complex formation.

Since the observed 'apparent' quenching constants for excimer fluorescence, k_e, are larger than the quenching constants for monomer fluorescence, k_m, it can safely be said that the quenching of pyrene excimer by these quenchers does occur.[17] Furthermore, the fact that $k_e < 2.4\,k_m$ shows that the excimer is less sensitive to the quenching action of these quenchers,[13] indicating that the electron-donating ability of excimer is smaller than that of the excited monomer.

15) Strictly speaking, the k-value for a quencher depends on its diffusion constant in solution. However, the differences among the diffusion constants of these quenchers are too small to change the order of k-values.

16) The complex emission of pyrene-tetrachlorophthalic anhydride was the mirror image of the corresponding charge transfer complex absorption.

17) A kinetic analysis gives the following relation:

$$k_e \geq k_m + k_{te}$$

where k_{te} is the 'true' quenching constant for excimer fluorescence, defined as an analogous expression to k_m.

In the case where no 'true' quenching of excimer occurs, $k_e = k_m$; otherwise holds the inequality. It can also be shown that when the quenching of excimer occurs to the same extent as the quenching of excited monomer,

$$k_e = (1 + \sqrt{2})k_m = 2.4\,k_m.$$