Fluorescence Quenching due to the Interactions between π-Electron Systems in the Excited State and Acid-Base Relation: Nitrogen Heterocycles, Naphthylamine and Naphthol in Aqueous and Alcoholic Solution

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In an earlier paper[1], we have reported the results of our investigation about the hydrogen bonding effect on the fluorescence of acridine, 3,6-diaminoacridine (DAA) and 3,6-bisdimethylaminoacridine (BDAA) in non-polar solvent mixed with proton donor such as phenol, naphthol, aniline and pyrrole. In these cases in which the hydrogen bond is capable of conjugating with the π-election system of proton donor, the fluorescence yields decrease as the result of hydrogen bonding formation in contrast to the case of donors such as benzylalcohol and β-phenylethylalcohol[2] in which the hydrogen bond is isolated by σ-bond from π-electron system, and with which hydrogen bonding leads to the enhancement of fluorescence yield. From the fact that no viscosity dependence of quenching has been observed, the possibility of the participation of diffusion-controlled dynamical process in the non-radiative degradation of excited state has been excluded. Moreover, we have also examined the case of naphthylamines and naphthol, hydrogen-bonded with pyridine where a remarkable fluorescence quenching has been observed[3]. Then the cause of the non-radiative degradation of excited state by means of hydrogen bonding has been attributed to the interaction between two π-electron systems via the hydrogen bond, viz., the delocalization of π-electrons through the hydrogen bond, especially in the excited state.

Hitherto, the ordinary experimental studies of the fluorescence quenching in the case of complex molecules, has been mainly carried out in aqueous or alcoholic solution, and the real nature of this phenomenon is not very clear up to now.

We have attempted to touch the real nature of the fluorescence quenching, in our series of investigation[1-7], mainly concerning with the case of hydrogen bond formation between fluorescer and quencher (or promoter) in non-polar solvent. The above mentioned case of fluorescence quenching due to the hydrogen bonding between the π-electron systems[1,3] may plausibly provide a more clear-cut example in relation to the mechanism of fluorescence quenching, and, in fact, it may be a new type of fluorescence quenching studied in a systematic manner. As an extention of our investigations, we have examined the behavior of the fluorescers and quenchers in aqueous and alcoholic solutions. We have found that, in this case, there exists some regularity in relation to the ability of a molecule as quencher or an approximate parallelism with the case of fluorescence quenching in non-polar solvent. This fact may probably suggest a somewhat analogous mechanism of quenching to the case of non-polar solvents, although, contrary to the case of the latter, the dynamical mechanism may be predominant in the present case. Moreover, the remarkable difference between aqueous and alcoholic solutions in this quenching phenomenon has become clear. In the present paper we shall report these facts with some discussions on the characterization of the quenchers and the influence of liquid structures of solvents on the fluorescence quenching.

Experimental

Apparatus: Absorption spectra were measured with Beckman Spectrophotometer model

1) N. Mataga and S. Tsuno, This Bulletin, **30**, 711 (1957).
2) N. Mataga and S. Tsuno, ibid., **30**, 368 (1957).
3) N. Mataga, to be published in this Bulletin.
4) N. Mataga, Y. Kaifu and M. Koizumi, *Nature*, **175**, 731 (1955); This Bulletin, **29**, 115 (1956).
5) N. Mataga and S. Tsuno. *Naturwiss.*, **44**, 304 (1957).
6) N. Mataga, to be published in this Bulletin.
7) N. Mataga, to be published in this Bulletin.

DU. Fluorometer was the same as described before[4]. A high pressure mercury lamp with appropriate filters was used as an exciting light source. Filters for quinoline, acridine[8] and DAA[1] are the same as used before. The filters used for β-naphthylmethyl ether and the ultraviolet fluorescence[9] of β-naphthol were the same as those of quinoline. For the blue fluorescence[9], the saturated solutions of nickel- and cobalt-sulfate and 0.2 g./l. potassium chromate solution each of which has 1 cm. thickness, were used as exciting light filters, taking out only 310 mμ line of mercury lamp, on the one hand, and yellow filter of Beckman photometer[3] was used for fluorescent light on the other hand. The filters used for β-naphthylamine and *N,N*-dimethyl-α-naphtylamine were the same as used before[3].

Reagent: All fluorescers and quenchers are the same samples as used before[1-5,8]. In order to control the pH of aqueous solution, citrate, phosphates and glycine were used. The reagents were of extra-pure grade, and used without further purification.

Experimental Results

In the following, the results for each fluorescer are described separately, and some of the data are collected in tables* in the latter part of this section. The concentrations of fluorescers are as follows; quinoline, β-naphthol, β-naphthylamine, β-naphylmethyl ether and *N,N*-dimethyl-α-naphthylamine, $\sim 10^{-4}$ mole/l.; acridine, 5×10^{-5} mole/l.; DAA, $\sim 10^{-5}$ mole/l.

In all cases described here, the absorption spectra showed no change caused by the addition of quenchers.

a) Quinoline: Only the quenching action of phenol and pyridine in aqueous solutions of appropriate pH values where the fluorescer exists exclusively as quinolinium ion, was examined quantitatively. The quenching curves in the case of phenol, are well reproduced by Stern-Volmer** equation, and the quenching constant becomes larger, with decrease in the pH value. The fluorescence is quenched also by pyrrole, almost completely disappearing at the quencher concentration, $\sim 10^{-1}$ mole/l. No quenching by pyridine, however, has been observed.

b) Acridine: The quenching actions of phenol, aniline pyrrole, and pyridine were examined mainly in aqueous solutions of various pH values. In the case of phenol, the dependence of quenching constant on the pH value is similar to the case of quinoline. Contrary to this, in the case of aniline, the quenching constant at pH 1 is the smallest. The quencher exists at this pH value, however, as anilinium cation. In contradistinction to the quenching in aqueous solution, the efficiency of phenol in ethanol solution is very poor, and, in practice, there occurs almost no quenching. The efficiencies of aniline and pyrrole in alcoholic solution, however, are not smaller than those in aqueous solution. Moreover, pyridine has no quenching effect on the fluorescence of acridine either in aqueous or alcoholic solutions. The anomalous behavior of phenol as quencher in alcoholic solution compared with the remarkable quenching in aqueous solution, has been studied in detail for the case of DAA.

c) DAA: Mainly the effect of solvent on the fluorescence quenching by phenol, aniline and pyrrole, has been examined in detail. The pH dependence of the quenching by phenol in aqueous solution is analogous to the case of acridine, although practically no quenching occurs at pH 13. In the case of aniline and pyrrole in aqueous solution, measurement was made only at pH 5. The remarkable difference between aqueous and alcoholic solutions is as follows. Although the quenching constants in the case of aniline and pyrrole in various alcoholic solvents are in approximately the same order of magnitude as those in aqueous solution, and the quenching in alcoholic solutions shows a normal dependence on the viscosity of solvent, all values of quenching constants in the case of phenol in alcoholic solutions are ca. 0.1 (l./mole), i.e., practically no quenching occurs in this case. Here also, pyridine shows no quenching effect on the fluorescence either in aqueous or alcoholic solutions.

d) β-Naphthol: In aqueous solution some of the excited naphthol molecules dissociate into ions during the lifetime of excited molecules[9]. Therefore, in this case, the fluorescence of molecule (ultraviolet) and that of ion (blue) should be measured separately. The formula representing the relative fluorescence intensities of molecule and ion, respectively are derived easily employing the following scheme.

8) N. Mataga, Y. Kaifu and M. Koizumi, This Bulletin, **29**, 373 (1956).

9) Th. Förster, *Z. Elektrochem.*, **54**, 42, 531 (1950); A. Weller, ibid., **56**, 662 (1952); *Z. physik. Chem.*, N. F., **3**, 238 (1955).

* In these tables, when the quenching curves show some deviations from Stern-Volmer equation, the values of quenching constants are extrapolated from the regions of dilute quencher concentrations.

** Hereafter, abbreviation "S.-V. equation" will be used.

TABLE I

NITROGEN HETEROCYCLES

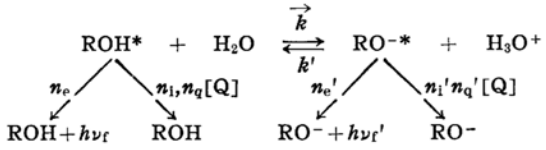
Quencher	Solvent (pH)	Quenching Constant (l./mole)*	c_h** (mole/l.)***	t(°C)****
		Quinoline		
	H_2O (1)	65	1.5×10^{-2}	~17
	H_2O (2.97)	32	3.1×10^{-2}	〃
		Acridine		
phenol	H_2O (2.97)	130 ($<2\times10^{-2}$)	7.7×10^{-5}	~20
	H_2O (6.81)	110 ($<3\times10^{-2}$)	9.7×10^{-3}	〃
	H_2O (7.73)	8 ($<4\times10^{-2}$)	1.5×10^{-2}	〃
	H_2O (11.3)	30 ($<4\times10^{-2}$)	3.6×10^{-2}	〃
	H_2O (12.9)	37 ($<5\times10^{-2}$)	3.0×10^{-2}	〃
aniline	H_2O (1.04)	10.6	9.4×10^{-2}	~14
	H_2O (7.17)	94.4	1.05×10^{-2}	〃
	H_2O (13)	32.3	3.09×10^{-2}	〃
pyrrole	H_2O (5)	77	1.3×10^{-2}	~14
		DAA		
phenol	H_2O (2.97)	21.6	4.6×10^{-2}	~21
	H_2O (4.96)	20.8	4.8×10^{-2}	〃
	H_2O (12.9)	no quenching		〃
	MeOH	0.13	(8.0)	~13
	EtOH	0.11	(9.0)	〃
	BuOH	0.12	(8.4)	〃
aniline	H_2O (5)	8	1.2×10^{-1}	~14
	MeOH	32	3.2×10^{-2}	〃
	EtOH	18	5.7×10^{-2}	〃
	BuOH	11.4	8.7×10^{-2}	〃
pyrrole	H_2O (5)	19	5.4×10^{-2}	~14
	MeOH	21	4.7×10^{-2}	〃
	EtOH	6.1	1.6×10^{-1}	〃
	BuOH	3.5	2.8×10^{-1}	〃

* An extrapolation was made in the range of the quencher concentration smaller than this value.

** The concentration of quencher at which f becomes $f_0/2$.

*** Extrapolated value in the case of very small quenching constant.

**** Temperature at which the measurement was made.



where $k'=\overleftarrow{k}[H_3O^+]$.

Then, for molecule,

$$f/f_0 = \frac{1+\alpha'[Q]}{1+\alpha[Q]+\beta[Q]^2}$$

and for ion,

$$f'/f_0' = \frac{1}{1+\alpha[Q]+\beta[Q]^2}$$

where Q represent quencher.

Therefore, $\left(\frac{f/f_0}{f'/f_0'}\right)=1+\alpha'[Q]$.

The meanings of constants α, α' and β are as follows,

$$\alpha=\frac{n\tau_q(1+k'\tau')+n_q'\tau'(1+\vec{k}\tau)}{1+\vec{k}\tau+k'\tau'}, \quad \alpha'=\frac{n_q'\tau'}{1+k'\tau'}$$

$$\beta=\frac{n_q n_q'\tau\tau'}{1+\vec{k}\tau+k'\tau'}$$

where τ and τ' are the mean lifetimes of excited molecule and ion, respectively. When $k'\sim0$, i.e., $[H_3O^+]$ is small, above equations become as follows.

$$f/f_0=\frac{1}{1+\{n\ \tau/(1+\vec{k}\tau)\}[Q]}$$

$$\left(\frac{f/f_0}{f'/f_0'}\right)=1+n_q'\tau'[Q]$$

These equations are well satisfied by the experimental observations. Using the experimentally datermined values of $\vec{k}$, τ and τ'[9], n_q and n'_q are evaluated. The values of n_q and n'_q are almost the same, although the latter is slightly larger than the former. The dependence of n_q and n'_q on the pH values of the solution is analogous to the case of nitrogen heterocycles as fluorescers and phenol as quencher, i.e., the n_q and n'_q values become larger, the smaller the pH value is. Moreover, also in this case, the quenching efficiency in alcoholic solution is very poor compared with that in aqueous solution.

e) **β-Naphthylamine:** Remarkable quenching by pyridine occurs in both aqueous and methanol solution, although the quenching constant in the latter solution is somewhat less prominent. The quenching curves are well satisfied by S.-V. equation. Contrary to this, phenol does not show any quenching effect, and the efficiency of aniline as quencher is negligibly small.

f) **β-Naphthylmethyl ether:** In a preceding paper[3], we have reported that, no quenching effect by pyridine in benzene solution on the fluorescence of this molecule has been observed. Here it is confirmed that also in cyclohexane and acetic acid esters, the same situation prevails. In aqueous and alcoholic solutions, however, there occurs considerable quenching, respectively, almost comparable with the corresponding cases of β-naphthol molecule.

g) ***N, N*-Dimethyl-α-naphthylamine:** The circumstance is analogous to the case of β-naphthylmethyl ether. In benzene solution, the fluorescence of this molecule is not influenced by pyridine[3]. In aqueous solution, however, the fluorescence is quenched by pyridine considerably.

TABLE II

β-NAPHTHOL-PYRIDINE

Solvent (pH)	n_q	n'_q	t(°C)
H_2O (3.95)	3.6×10^9	4.2×10^9	~16
H_2O (7.17)	1.7×10^9	3.1×10^9	〃

TABLE III

β-NAPHTHYLAMINE-PYRIDINE

Quencher	Solvent	Quenching Constant	t(°C)
pyridine	H_2O*	63	~16
	MeOH	26	〃

*pH is not controlled by buffer.

Discussion and Concluding Remarks

In previous papers[2,6,7], we have reported the fact that, in the case of nitrogen heterocycles, the difference in the emitting π-electronic state leads to the different behavior of these nitrogen heterocycles in fluorescence quenching by the near-by existence of halogen atoms. Some qualitative considerations by means of analogy to the π-electronic structures of parent hydrocarbons were given[2], and a quantitative argument has been given on the basis of a theoretical computation[6,7]. In the present case, however, no such effect of emitting π-electonic state on the fluorescence quenching is observed, and phenol quenches the fluorescence of quinoline as well as acridine and DAA almost equally well. Therefore, with the present type of fluorescence quenching which is caused by the interaction between π-electron systems in the excited state, ^3L_b state may not concern so severely, although the detailed microscopic mechanism of the quenching is not so clear. Now, in the following, some arguments will be given from two standpoints, i.e., the most effective combination of fluorescer and quencher, on the one hand, and the effects of solvents on the quenching efficiency, on the other hand.

A. Fluorescence Quenching and Acid-Base Relation. Phenol, aniline and pyrrole quench severely the fluorescence of nitrogen heterocycles such as quinoline, acridine and DAA. Contrary to this, pyridine does not show any quenching effect on the fluorescence of the nitrogen heterocycles.

Moreover, the fluorescence of naphthol as well as naphthylamine is quenched by pyridine effectively. Phenol and aniline, however, scarcely quench the fluorescence of these naphthalene derivatives at all. Thus, it is evident that the most efficient fluorescer-quencher pair is in acid-base or proton donor-acceptor relation, and roughly speaking, there exists a parallelism between the fluorescence quenching due to hydrogen bonding in non-polar solvent and the present case.

From this fact, we might be allowed to suppose that some analogous mechanism to the case of fluorescence quenching due to hydrogen bonding in non-polar solvent[1],

may prevail also in the quenching of present type, although the circumstance is more complicated.

B. Effects of Solvents on the Efficiency of Fluorescence Quenching. Pyridine does not quench the fluorescence of β-naphthylmethyl ether as well as *N,N*-dimethyl-α-naphthylamine, in benzene, cyclohexane and acetic acid esters. In aqueous and alcoholic solutions where the hydrogen bond between solvent molecules and also between solvent and solute molecules exist, however, the fluorescence of these molecules is quenched remarkably to almost the same extent as in the case of naphthol and naphthylamine, respectively. The important role of hydrogen bonding in the fluorescence quenching of this case is implicit in the fact that, only in the associating liquids such as water and alcohol, the fluorescence is quenched efficiently.

Another remarkable example of the solvent effects is that the behavior of the phenol-nitrogen heterocycles or pyridine-naphthol system in alcoholic solution is very different from that in aqueous solution.

In contradistinction to the severe quenching in aqueous solution, the quenching in alcoholic solution is very slight. Although the cause of this remarkable difference is not clear, we can not but ascribe it to the difference in the liquid structure, viz., the very extensive hydrogen bonding in water on the one hand and the less extensive hydrogen bonding in alcohol on the other hand might have some connection with this phenomenon.

Summary

The acid-base relation in the fluorescence quenching in aqueous and alcoholic solutions, due to the interaction between π-electron systems in the excited state, has been pointed out. Roughly speaking, this is parallel with the fluorescence quenching due to hydrogen bonding between the π-electronic fluorescer and quencher in non-polar solvent. Remarkable solvent effects on the quenching efficiency have been found and some important roles of hydrogen bonding in this phenomenon have been suggested.

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