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Fluorescence Quenching of *N*-Phenyl-1-naphthylamine by Nitriles, Esters, and Amines

Sanyo HAMAI
Department of Physics, Miyazaki Medical College, Kiyotake, Miyazaki 889-16
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The equilibrium constants for the hydrogen bonding between *N*-phenyl-1-naphthylamine (NPNA) and proton acceptors (1,4-dioxane, nitriles, esters, and amines) in cyclohexane and the corresponding thermodynamic parameters have been determined. The fluorescence of NPNA is quenched by nitriles, esters, and amines. In the cases of nitriles and esters, the quenching is caused by compounds in which a cyano or carboxyl group is conjugated with a double bond. The rate constants for quenching of the NPNA fluorescence by methyl tiglate, methyl 3,4,5-trimethoxybenzoate, and 2,4,6-trimethylpyridine are 81, 73, and 69% of those by methyl acrylate, methyl benzoate, and pyridine, respectively. These findings can be interpreted in terms of the charge-transfer interaction through the hydrogen bond between NPNA (an electron donor) and the proton acceptors (electron acceptors) except for the amines, for which a mechanism different from the charge-transfer mechanism may be important in explaining the fluorescence quenching.

It is well-known that 1- or 2-naphthylamine is hydrogen-bonded to pyridine etc.[1-3] It has been shown that when two conjugated π-electronic systems are directly combined by the hydrogen-bonding interaction, the fluorescence of 1- or 2-naphthylamine is quenched.[1-4] As the quenching mechanism in these cases, a charge-transfer interaction between a proton donor and a proton acceptor through the hydrogen bond has been suggested.[1-5]

Derivatives of *N*-phenyl-1-naphthylamine (NPNA), 1,8-ANS (8-anilino-1-naphthalenesulfonate) etc., are frequently used as fluorescent probes in the areas of biochemistry and biology.[6,7] However, the hydrogen-bonding interaction between a fluorescent probe and substrates has not been noted, although these probes may be hydrogen bonded to substrates. Thus, we studied the quenching behavior of the fluorescence of NPNA in cyclohexane by using additives forming a hydrogen bond with NPNA.

Experimental

N-Phenyl-1-naphthylamine (NPNA) was recrystallized from cyclohexane three times. Ethers, nitriles, esters, and amines were distilled under atmospheric or reduced pressure except for methyl 3,4,5-trimethoxybenzoate and 1,4-diazabicyclo[2.2.2]octane (DABCO) which were recrystallized twice from ethanol and hexane, respectively. Cyclohexane was purified by percolation through a silica-gel column. Absorption spectra were recorded with a Shimadzu UV-260 spectrophotometer. Fluorescence spectra were taken with a Shimadzu RF-501 spectrofluorometer. Fluorescence spectra were corrected for the spectral response of the spectrofluorometer.[8] The fluorescence lifetime of NPNA was measured with an Ortec 9200 nanosecond decay time fluorimeter equipped with a Horiba NFL-111A ns lamp. Fluorescence quantum yields were determined relative to a quantum yield of 0.90 of 9,10-diphenylanthracene in nitrogenated cyclohexane.[9] The concentrations of NPNA for the measurement of absorption and fluorescence were 10^{-4} and 3×10^{-5} mol dm^{-3}, respectively. Sample solutions for the fluorescence measurement were nitrogen bubbled. Unless otherwise stated, all the measurements were made at 25±0.1 °C.

Results and Discussion

Figure 1 shows the absorption spectra of NPNA in cyclohexane in the absence and presence of 1,4-dioxane. The absorption maximum of NPNA is shifted to the red with isosbestic points at 284 and 332 nm. These spectral changes indicate a hydrogen bonding between NPNA and 1,4-dioxane. In the hydrogen-bonding complex with 1,4-dioxane, a proton donor is NPNA and a proton acceptor 1,4-dioxane. From the spectral changes of NPNA in Fig. 1, an equilibrium constant (K_g) for the hydrogen bonding in the ground state could be evaluated, and its value is shown in Table 1 together with the enthalpy change (ΔH) and the entropy change (ΔS) of K_g which were determined from the temperature dependence of K_g.[10] For nitriles, esters, and amines, similar changes to those for 1,4-dioxane in the absorption spectra of NPNA were observed, indicating a hydrogen bonding with NPNA.

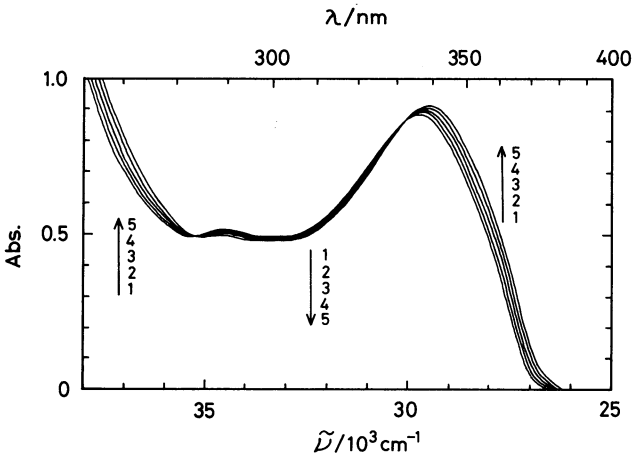

Fig. 1. Absorption spectra of NPNA (10^{-4} mol dm^{-3}) in cyclohexane in the presence of 1,4-dioxane. Concentration of 1,4-dioxane: (1) 0 mol dm^{-3}; (2) 0.117 mol dm^{-3}; (3) 0.234 mol dm^{-3}; (4) 0.585 mol dm^{-3}; (5) 1.17 mol dm^{-3}.

Table 1. Equilibrium Constants for Hydrogen Bonding of NPNA in the Ground State at 25 °C (K_g), Enthalpy Changes (ΔH), and Entropy Changes (ΔS)

	K_g/mol^{-1} dm^3	ΔH/kJ mol^{-1}	ΔS/J K^{-1} mol^{-1}
1,4-Dioxane	1.8	−26	−84
Propionitrile	2.5	−10	−27
Acrylonitrile	1.6	−12	−38
Benzonitrile	2.0	−12	−33
Ethyl acetate	1.8	−17	−50
Methyl acrylate	1.2	−18	−58
Methyl tiglate	1.4	−22	−72
Dibutyl oxalate	2.5	−21	−63
Dibutyl maleate	4.1	−21	−60
Methyl benzoate	1.4	−20	−63
Methyl 3,4,5-trimethoxybenzoate	6.3	−17	−42
Pyridine	3.2	−8.8	−20
2,4,6-Trimethylpyridine	5	—	—
Triethylamine	0.3	−35	−130
DABCO	2	−42	−130

The compounds are proton acceptors like 1,4-dioxane for NPNA. The values of K_g, ΔH, and ΔS for these esters, nitriles, and amines are also given in Table 1. As can be seen from Table 1, similar values of K_g are obtained for each group of proton acceptors except for dibutyl oxalate, dibutyl maleate, methyl 3,4,5-trimethoxybenzoate, and triethylamine. About twofold larger values of K_g for dibutyl oxalate and dibutyl maleate than those for monocarboxylates (ethyl acetate, etc.) can be explained by the fact that two carboxyl groups are involved in one molecule. The large difference in K_g between triethylamine and DABCO may be partly due to the difference of the number of the amino nitrogen. The larger value of K_g for methyl 3,4,5-trimethoxybenzoate or 2,4,6-trimethylpyridine than that for methyl benzoate or pyridine can be interpreted in terms of an increase of the electron density on a carboxyl oxygen atom or a nitrogen atom caused by an electron-donating substituent (methoxy or methyl group). The values for ΔH and ΔS are not too different within the same groups of acceptors.

The fluorescence spectra of NPNA in cyclohexane in the presence of 1,4-dioxane are shown in Fig. 2. When 1,4-dioxane is added to an NPNA solution, the fluorescence maximum of NPNA is shifted to longer wavelengths with an isoemissive point at 388 nm. An enhancement of the integrated fluorescence intensity is also observed. These spectral changes can be ascribed to the hydrogen bonding between NPNA and 1,4-dioxane. The enhancement of the NPNA fluorescence indicates that the fluorescence quantum yield (ϕ_f) is larger in a hydrogen-bonding form of NPNA than in a free form.

We examined the solvent dependence of ϕ_f and the fluorescence maximum position of NPNA. The values of ϕ_f in cyclohexane (ε=2.02), 1,4-dioxane (ε=2.21), ethyl acetate (ε=6.02), propionitrile (ε=27.2),

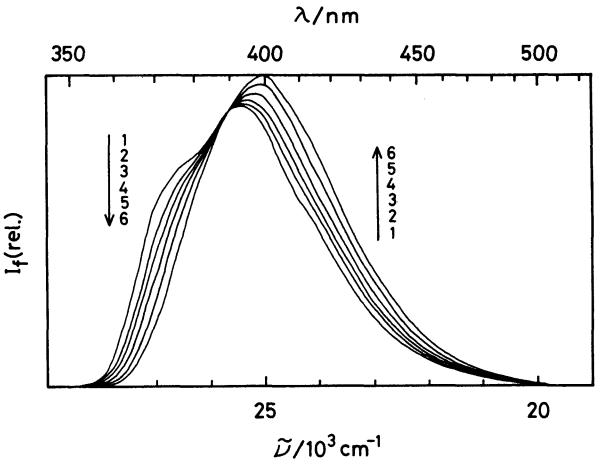

Fig. 2. Fluorescence spectra of NPNA (3×10^{-5} mol dm^{-3}) in cyclohexane in the presence of 1,4-dioxane. Concentration of 1,4-dioxane: (1) 0 mol dm^{-3}; (2) 0.059 mol dm^{-3}; (3) 0.117 mol dm^{-3}; (4) 0.234 mol dm^{-3}; (5) 0.585 mol dm^{-3}; (6) 1.17 mol dm^{-3}. λ(exc.)=332 nm.

and water (ε=78.5) were 0.68, 0.72, 0.63, 0.54, and 0.029, respectively. The larger the dielectric constant of the solvent, the smaller ϕ_f of NPNA except for 1,4-dioxane. The dielectric constant of 1,4-dioxane (2.21) is very close to that of cyclohexane (2.02). In the case of 1,4-dioxane, therefore, the effect of the hydrogen bonding, which enhances the fluorescence intensity, prevails over the effect of the solvent polarity. The fluorescence maximum in cyclohexane, 1,4-dioxane, ethyl acetate, propionitrile, and water are at 393, 413, 420, and 485 nm, respectively. The maximum position is red-shifted as the solvent polarity is increased. Such characteristics of both ϕ_f and the fluorescence peak have been reported for other naphthylamine derivatives.[11,12]

Figure 3 shows the fluorescence spectra of NPNA solutions containing varying concentrations of propionitrile. Below ≈0.07 mol dm^{-3} of propionitrile, the spectral change, with an isoemissive point at 389 nm, is similar to that for 1,4-dioxane (Fig. 2). Although the dielectric constant of propionitrile (27.2) is very large compared with that of cyclohexane (2.02), the polarity of the cyclohexane solution containing such a low concentration of propionitrile is not too different from that of neat cyclohexane. Above ≈0.14 mol dm^{-3} of propionitrile, the fluorescence intensity is reduced, accompanied by a red shift of the fluorescence maximum; also, the isoemissive point at 389 nm disappears. In the high-concentration range of propionitrile, the solvent polarity becomes fairly high. Thus, the effect of solvent polarity which decreases the fluorescence intensity overcomes the effect of the hydrogen bonding to decrease the fluorescence intensity.

In Table 2 are summarized the proton acceptors by which the fluorescence quenching of NPNA is caused (O) or not caused (X). The ethers examined in this study show no fluorescence quenching. In the nitrile series, acrylonitrile and benzonitrile quench the NPNA fluorescence, while acetonitrile and propionitrile do not quench. Acrylonitrile and benzonitrile have a double bond which is conjugated with a cyano group although in benzonitrile a conjugated double bond belongs to a benzene ring. For the fluorescence quenching of naphthylamines, quenchers forming a hydrogen bond are pyridine and quinoline whose conjugated double bond is present as a part of an aromatic ring.[1-3] In the case of acrylonitrile, however, a cyano group is conjugated with a nonaromatic double bond. In the ester series, ethyl acetate and vinyl acetate do not quench the NPNA fluorescence. However, the

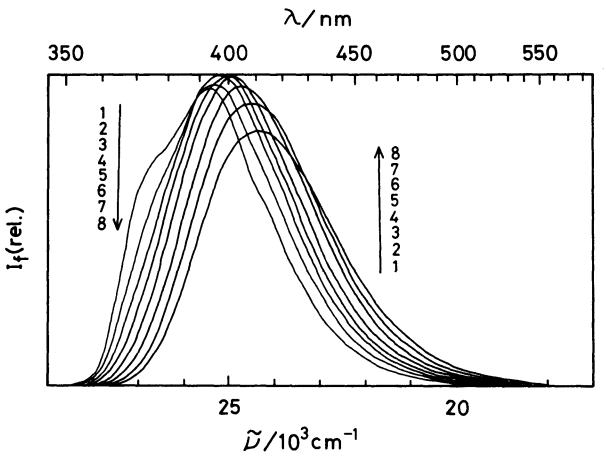

Fig. 3. Fluorescence spectra of NPNA (3×10^{-5} mol dm^{-3}) in cyclohexane in the presence of propionitrile. Concentration of propionitrile: (1) 0 mol dm^{-3}; (2) 0.028 mol dm^{-3}; (3) 0.071 mol dm^{-3}; (4) 0.142 mol dm^{-3}; (5) 0.284 mol dm^{-3}; (6) 0.710 mol dm^{-3}; (7) 1.42 mol dm^{-3}; (8) 2.84 mol dm^{-3}. λ(exc.)=326 nm.

Table 2. Proton Acceptors Which Exhibit Quenching (O) or No Quenching (X) for the NPNA Fluorescence

Proton acceptor	Result
1,4-Dioxane	X
Ethyl vinyl ether	X
Furan	X
Acetonitrile	X
Propionitrile	X
Acrylonitrile	O
Benzonitrile	O
Ethyl acetate	X
Vinyl acetate	X
Methyl acrylate	O
Methyl tiglate	O
Dibutyl oxalate	O
Dibutyl maleate	O
Methyl benzoate	O
Methyl 3,4,5-trimethoxybenzoate	O
Pyridine	O
2,4,6-Trimethylpyridine	O
Triethylamine	O
DABCO	O

fluorescence quenching takes place by methyl acrylate, methyl tiglate, dibutyl oxalate, dibutyl maleate, methyl benzoate, and methyl 3,4,5-trimethoxybenzoate. These esters also possess a double bond which is conjugated with a carboxyl group. For methyl acrylate, methyl tiglate, dibutyl oxalate, and dibutyl maleate, the double bonds are nonaromatic ones. In the case of amines, pyridine and 2,4,6-trimethylpyridine are effective quenchers for the NPNA fluorescence. Besides pyridine and 2,4,6-trimethylpyridine, aliphatic amines (triethylamine and DABCO) were found to quench the fluorescence.

All the quenchers shown in Table 2 are hydrogen bonded to NPNA in the ground state. Because the K_g values for these quenchers are not so large (1.2—6.3 mol^{-1} dm^3), the concentration of a hydrogen-bonding complex can be neglected in the low-concentration range of the quencher. Thus, the fluorescence quenching by these proton acceptors was analyzed according to the Stern-Volmer plot. From the Stern-Volmer quenching constants and the fluorescence lifetime of NPNA (3.9 ns), the rate constants for fluorescence quenching could be determined and are listed in Table 3.[13] These values in Table 3 represent the rate constants for the dynamic quenching in the excited state of NPNA. Almost diffusion-controlled rate constants are obtained for the nitriles. Although the quenchers shown in Table 3 can be hydrogen bonded to excited NPNA, the nonfluorescent exciplex formation may be possible for benzonitrile, methyl benzoate, and methyl 3,4,5-trimethoxybenzoate. Thus, we investigated the fluorescence quenching of *N*,*N*-dimethyl-1-naphthyl-

Table 3. Rate Constants for Fluorescence Quenching of NPNA (k_q)

Proton acceptor	$k_q/10^{10}$ mol^{-1} dm^3 s^{-1}
Acrylonitrile	1.60
Benzonitrile	1.53
Methyl acrylate	1.17
Methyl tiglate	0.95
Dibutyl oxalate	1.08
Dibutyl maleate	1.42
Methyl benzoate	1.14
Methyl 3,4,5-trimethoxybenzoate	0.83
Pyridine	1.19
2,4,6-Trimethylpyridine	0.82
Triethylamine	0.28
DABCO	1.00

amine (DMNA) by these proton acceptors. Its oxidation potential (0.46 V), estimated from the fluorescence maximum position of the perylene exciplexes with DMNA, *N,N*-diethylaniline, and *N*-ethylcarbazole, is much smaller than that of NPNA estimated in a similar fashion (0.89 V).[14,15] In addition, DMNA cannot be hydrogen bonded to proton acceptors in contrast to NPNA. It was found that no fluorescence quenching of DMNA took place by the above proton acceptors which quenched the NPNA fluorescence. From this finding, it is clearly evident that NPNA does not form a nonfluorescent exciplex with the above proton acceptors. Therefore, the hydrogen bonding in the excited state of NPNA is responsible for the fluorescence quenching. As shown in Table 3, the quenching constants of methyl tiglate, methyl 3,4,5-trimethoxybenzoate, and 2,4,6-trimethylpyridine are about 81, 73, and 69% of those for methyl acrylate, methyl benzoate, and pyridine, respectively, indicating a reduction of the quenching constant by the substitution of an electron-donating group (methyl or methoxy group) in a proton acceptor. Therefore, the quenching is caused by the charge-transfer interaction between NPNA and proton acceptors through the hydrogen bond. In these cases, NPNA is an electron donor and the proton acceptors electron acceptors.

For a 2-naphthylamine-pyridine system, Ikeda et al. has successfully detected a transient absorption of a charge-transfer state from excited 2-naphthylamine to hydrogen-bonded pyridine.[5] Our results that the fluorescence of NPNA is quenched only by the proton acceptors possessing a double bond which is conjugated with a functional group and that NPNA acts as an electron donor in the fluorescence quenching are consistent with the observations for the 2-naphthylamine-pyridine system. The findings that ethers, nonconjugated nitriles and esters do not quench the NPNA fluorescence can also be interpreted in terms of the charge-transfer interaction through a hydrogen bond.

Since the aliphatic amines (triethylamine and DABCO) were found to quench the perylene fluorescence to a smaller extent than NPNA does, they seem to be weaker electron donors than NPNA. If so, it is difficult to attribute the fluorescence quenching of NPNA by these amines to their electron donating nature. Other mechanisms, such as a proton transfer or a hydrogen atom transfer, may be responsible for the fluorescence quenching by the aliphatic amines.

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