Fluorescence-Quenching Mechanism of Tryptophan. Remarkably Efficient Internal Proton-Induced Quenching and Charge-Transfer Quenching[1]

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Abstract: The fluorescence quenching of tryptophan (Trp) in the absence and presence of 18-crown-6 in CH_3OH–H_2O (9:1, v/v) mixtures has been studied by means of nanosecond time-resolved single-photon counting, fluorimetry, and photochemical H–D isotope exchange. The fluorescence intensity increases markedly with increasing concentration of 18-crown-6. The fluorescence quenching of Trp is not due to external quenching, but to internal quenching. The rate constant k_q for the internal quenching can be estimated from the equation $k_q = \tau_1^{-1} - (\tau_2^{max})^{-1}$, where τ_1 and τ_2^{max} denote the fluorescence lifetimes for free Trp and the 1:1 Trp–18-crown-6 complex, respectively. The internal quenching originates from the electrophilic protonation of the $^+NH_3$ (or $^+ND_3$) group of Trp at the C-4 position of the excited indole ring plus the charge-transfer interaction between the excited indole ring and the ammonium group. The stabilization constant K_g for the 1:1 complex of Trp with 18-crown-6 has been determined by means of fluorimetry.

The mechanistic study of the fluorescence decay of tryptophan (Trp) in polar media has been an interesting subject in photophysics and biophotochemistry.[3,4] A number of mechanisms for the decay process in the excited singlet state of Trp have been proposed.[3] Two types of quenching mechanisms have been proposed: internal and external, as described below. The internal quenching of Trp has been attributed to (a) simultaneous emission from uncoupled ^1L_a and ^1L_b[5] states (this interpretation was later discarded by the authors), (b) intramolecular charge-transfer quenching due to the interaction between the excited indole group and the alanyl side chain,[6-8] (c) internal charge-transfer quenching arising from different ground-state rotamers (the conformer model[9]) or from the modified conformer model containing both C_α–C_β and C_β–C_γ rotamers in the ground state,[10] and (d) proton-transfer quenching by the ammonium group.[11-16] Recently, Saito et al.[15,16] have shown by a photochemical H–D isotope-exchange reaction of Trp that the proton-transfer quenching occurs mainly at the C-4 position of the indole ring. The external quenching mechanism was assumed to be caused by the formation of an exciplex between the excited indole group and a polar solvent molecule[17,18] and by the charge transfer to solvent (CTTS)[19] or photoelectron ejection[20] of the excited indole moiety.

The fluorescence of Trp shows nonexponential decay in polar media,[9,10,14,19,21] suggesting that the rate for rotamer interconversion (rotational conversion) is comparable to that for the fluorescence decay. In a previous study,[22] we have shown that the relatively fast fluorescence decay of tryptamine is caused completely by internal quenching via the electrophilic protonation by the ammonium ion at the C-4 position of the excited indole ring. It is known that the proton-induced fluorescence quenching of aromatic compounds plays a very important role for excited-state acid–base reactions.[23] The proton-induced quenching is caused by electrophilic protonation at one of the carbon atoms of the excited aromatic ring leading to hydrogen exchange (or isotope exchange).[24] The complex formation of the ammonium ion with 18-crown-6 gives rise to interesting features in photochemical and photophysical properties of aromatic compounds with ammonium substituents.[25-27]

In the course of a study on the quenching mechanism of aromatic compounds, we were interested in the fluorescence-quenching mechanism of Trp. This paper concerns the fluorescence-quenching mechanism of Trp studied by means of nanosecond time-resolved single-photon counting and fluorimetry with the aid of the complex formation with 18-crown-6 and a photochemical H–D isotope exchange reaction. The following questions were asked: (1) Does Trp complex with 18-crown-6? (2) What effects are expected for the 18-crown-6 complex on the fluorescence decay (or fluorescence intensity) of Trp? (3) Which is the fluorescence quenching mechanism of Trp, the internal or the external one? Is it possible to estimate the absolute quenching

(1) The preliminary work was presented at the 54th Annual Meeting of the Chemical Society of Japan, Tokyo, April 1987. This work was supported by a Scientific Research Grant-in-Aid from the Ministry of Education, Science and Culture of Japan (No. 62113007). I.S. is grateful to the Ministry of Education, Science and Culture of Japan, Grant-in-Aid for Specially Promoted Research (No. 61065003).

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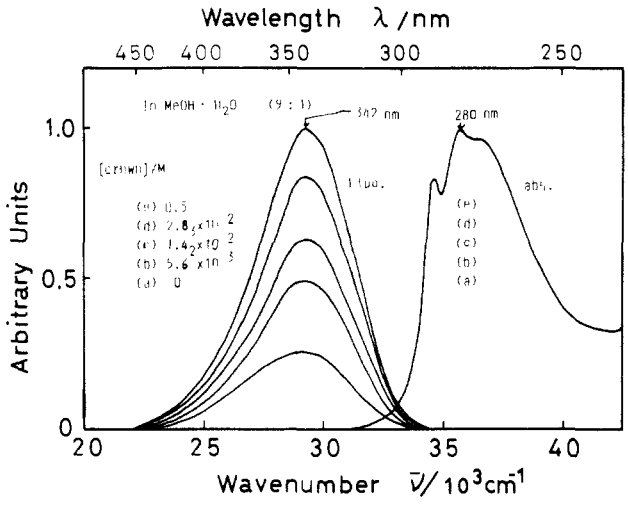


Figure 1. Concentration effect of 18-crown-6 on the absorption and fluorescence spectra of Trp in MeOH–H_2O (9:1) at 300 K.

rate? (4) What is the actual quenching mechanism of Trp?

Experimental Section

Tryptophan (Trp) from Wako was purified by recrystallizations from methanol. Deuteriated tryptophan (TrpD) was prepared by treating Trp with a 1 M NaOD–D_2O solution followed by titration with 0.1 M D_2SO_4. 18-Crown-6 (Merck) was purified by repeated recrystallizations from dichloromethane. Methanol (Spectrosol, Wako) and distilled water were used as a MeOH–H_2O mixture (9:1 by volume). For H–D isotope effect measurements, CH_3OD (Merck, 99%) and D_2O (Merck, 99.75%) were used as a MeOD–D_2O mixture (9:1 by volume). The concentration of Trp was $4.6_2 \times 10^{-5}$ M, and for 18-crown-6 the concentration was varied in the range 0–0.5 M. All sample solutions were thoroughly degassed by freeze–pump–thaw cycles on a high-vacuum line.

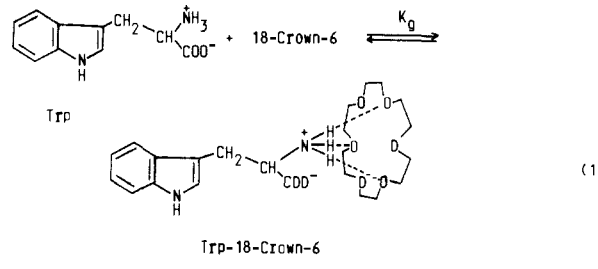
Absorption and fluorescence spectra were measured with Hitachi 139 and 200 spectrophotometers and a Hitachi M850 fluorimeter, respectively. Spectral corrections in emission were made. The fluorescence response functions were recorded with a nanosecond time-resolved spectrophotometer (Horiba NAES-1100, 2-ns pulse width). This single-photon-counting apparatus is able to measure both the exciting pulse and emission response functions simultaneously and to compute the decay parameters (up to triple-decay components) by the deconvolution method.

The photochemical H–D isotope-exchange reaction was carried out at 254 nm (using a low-pressure mercury lamp, Toshiba, 80 W) with a Vycor glass filter as well as by the procedure reported elsewhere.[24,28] Actinometry at 254 nm was made by using a ferric oxalate solution.[28] The assignment of the aromatic protons of Trp was made by the NOE technique, using 400-MHz ^1H NMR (JEOL JNM-GX400) as reported previously.[15,16]

Results and Discussion

Absorption and Fluorescence Spectra of Tryptophan in the Presence of 18-Crown-6. Figure 1 shows the absorption and fluorescence spectra of Trp ($4.6_2 \times 10^{-5}$ M) in the absence (a) and presence (b–e) of 18-crown-6 in MeOH–H_2O (9:1) mixtures at 300 K. It is well-known that the alanyl side chain of Trp has the ionic form of $CH_2CH(COO^-)^+NH_3$ at pH 7.[3] The absorption spectrum of Trp is very similar to that of indole or tryptamine, indicating that Trp has the π-isoelectronic structure seen with indole or tryptamine. This means that the side chain scarcely affects the π-electronic structure of the indole chromophore. No spectral change in absorption at 280 nm in the absence and presence of 18-crown-6 was observed, as can be seen in Figure 1. The fluorescent state of Trp, regardless of the presence or absence of 18-crown-6, is ascribed to the ^1L_a state, since the rapid relaxation in the excited singlet state of Trp produces the ^1L_a state in polar media.[19] There is no absorption due to 18-crown-6 itself at wavelengths longer than ~250 nm.[25-27]

However, the fluorescence intensity at 342 nm increased markedly with an increase of the concentration of 18-crown-6 without any spectral change, as shown in Figure 1. This observation suggests that the ammonium group of Trp plays an important role in the internal quenching of Trp. Similar phenomena have been observed in the tryptamine ammonium ion–18-crown-6 system.[22] The fluorescence enhancement is ascribable to the formation of the 1:1 complex Trp–18-crown-6 (eq 1). The ammonium ion complexed with 18-crown-6 cannot interact intramolecularly with the excited indole moiety R*. As a result, the internal fluorescence quenching of Trp decreases considerably as discussed later.



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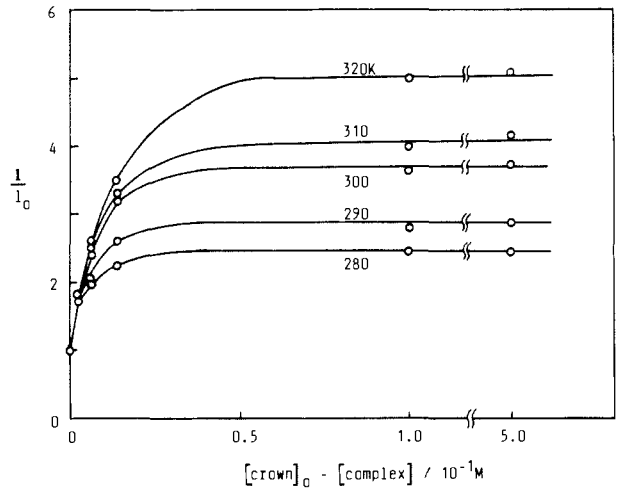

Figure 2. Concentration effect of 18-crown-6 upon the fluorescence intensity ratio (I/I_0) in MeOH–H_2O (9:1) at several temperatures. I and I_0 represent the fluorescence intensities with and without 18-crown-6, respectively.

Table I. Fluorescence Intensity Ratio I/I_0 as a Function of the Concentration of 18-Crown-6 in CH_3OH–H_2O (9:1) at Various Temperatures[a]

	I/I_0 at T (K)				
[crown], M	280	290	300	310	320
0	1.0	1.0	1.0	1.0	1.0
$1.4_2 \times 10^{-3}$	1.3_3	1.3_6	1.3_6	1.3_7	1.3_8
$2.8_3 \times 10^{-3}$	1.7_3	1.8	1.8_1	1.8_2	1.8_3
$7.0_8 \times 10^{-3}$	1.9_6	2.3	2.4	2.4_3	2.4_5
$1.4_2 \times 10^{-2}$	2.4_5	2.7_9	3.2_1	3.3_4	3.5_1
1.0×10^{-1}	2.2_4	2.6_5	3.7_3	4.0_6	5.0_6
5.0×10^{-1}	2.4_8	2.9_7	3.8_1	4.3_3	5.2_6

[a] Errors within 5%.

Figure 2 shows the plots of the fluorescence intensity ratio I/I_0 of Trp [$4.6_2 \times 10^{-5}$ M in CH_3OH–H_2O (9:1)] as a function of [18-crown-6] at several temperatures, where I and I_0 are fluorescence intensities at 342 nm with and without 18-crown-6, respectively. The I/I_0 value increases significantly at concentrations lower than ca. 0.02 M and approaches a maximum value at higher concentrations, as illustrated in Figure 2. The maximum values of I_{max}/I_0, which are temperature dependent, were obtained as 2.4_8 (280 K), 2.9_7 (290 K), 3.8_1 (300 K), 4.3_3 (310 K), and 5.2_6 (320 K) at [crown] = 0.5 M in CH_3OH–H_2O (9:1). These data are listed in Table I.

Determination of Association Constant K_g in the Ground State of the Tryptophan–18-Crown-6 Complexes. It is well-known that a 1:1 complex of organic (or inorganic) ammonium ion is readily

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Table II. Association Constants (K_g) and Thermodynamic Parameters (ΔG, ΔH, ΔS) of the 1:1 Trp–18-Crown-6 Complex in CH_3OH–H_2O (9:1)[a]

compd	$K_g/10^2$ M^{-1} at T (K)					ΔG,[b] kcal mol^{-1}	ΔH, kcal mol^{-1}	ΔS, eu
	280	290	300	310	320			
Trp	2.6_1	1.7_3	1.4_2	1.1_5	1.0_1	−3.0	−3.4	−1.4
tryptamine[c]	$44._1$	$24._4$	$17._8$	$12._5$	$10._7$	−4.5	−6.3	−5.9

[a] Errors with 5%. [b] At 300 K. [c] Data taken from ref 22.

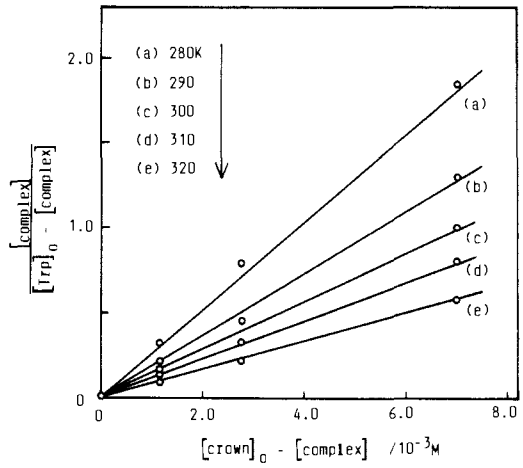

Figure 3. Plots of [complex]([Trp]$_0$ − [complex])$^{-1}$ as a function of ([crown]$_0$ − [complex]) at several temperatures.

formed with 18-crown-6.[23,25-27,29-31] The association constants for some ammonium ion–18-crown-6 systems have been determined by means of fluorimetry.[22,25-27] In the present system, the fluorimetric method was employed to determine the association constant K_g between Trp and 18-crown-6. The concentration of the Trp–18-crown-6 complex in the ground state is given by[22,25-27] eq 2, where I and I_0 denote the fluorescence intensities of Trp at

$$[\text{complex}] = \frac{(I/I_0) - 1}{(I_{max}/I_0) - 1}[\text{Trp}]_0 \qquad (2)$$

342 nm with and without 18-crown-6, respectively, I_{max} is the maximum fluorescence intensity of Trp in the presence of a sufficient concentration of 18-crown-6 (0.5 M), and $[\text{Trp}]_0$ represents the concentration of the added Trp ($4.6_2 \times 10^{-5}$ M).

According to the law of mass action, the association constant K_g for the 1:1 complex between Trp and 18-crown-6 in the ground state is expressed by eq 3, where $[\text{crown}]_0$ represents the con-

$$K_g = \frac{[\text{complex}]}{([\text{Trp}]_0 - [\text{complex}])([\text{crown}]_0 - [\text{complex}])} \qquad (3)$$

centration of 18-crown-6 added to the system. Equation 4 is,

$$\frac{[\text{complex}]}{[\text{Trp}]_0 - [\text{complex}]} = K_g([\text{crown}]_0 - [\text{complex}]) \qquad (4)$$

therefore, derived from eq 3. The plots of [complex]([Trp]$_0$ − [complex])$^{-1}$ as a function of ([crown]$_0$ − [complex]) at several temperatures are depicted in Figure 3, each of which gives a straight line. The experimental results are in good agreement with eq 4. The K_g values in CH_3OH–H_2O (9:1) were determined to be $2.6_1 \times 10^2$ (280 K), $1.7_3 \times 10^2$ (290 K), $1.4_2 \times 10^2$ (300 K), $1.1_5 \times 10^2$ (310 K), and $1.0_1 \times 10^2$ M^{-1} (320 K). The value of K_g for Trp is ~1 order of magnitude smaller than that for tryptamine. The presence of the bulky carboxylate group of Trp sterically hinders the complex formation with 18-crown-6. It has been already demonstrated that the K_g value is highly sensitive to the steric interaction between the aromatic moiety and 18-crown-6.[22,25-27,30] The cationic ammonium group of the complex seems to be displaced by about 1 Å from the mean oxygen plane of 18-crown-6, as shown by Nagano et al.[32] Therefore, the steric interaction in the complex decreases the K_g value significantly. From the linear van't Hoff plot of log K_g vs T^{-1}, the values of thermodynamic parameters of the complex, the free energy change (ΔG), enthalpy change (ΔH), and entropy change (ΔS) in CH_3OH–H_2O (9:1) were determined to be −3.0 kcal mol^{-1} at 300 K, −3.4 kcal mol^{-1}, and −1.4 eu, respectively. The values of ΔH and ΔS for the Trp–crown system are less negative than those of the tryptamine ammonium ion–18-crown-6 system, especially for ΔS, as shown in Table II. The ΔH value may reflect the steric hindrance due to the carboxylate group of Trp as stated above. The less negative ΔS value (−1.4 eu) is explained in terms of the difference in solvation between free Trp and Trp–crown complex; free Trp may be readily solvated in highly polar media such as CH_3OH–H_2O (9:1), since both $^+NH_3$ and COO^- groups of Trp are prone to be solvated, whereas in the Trp–crown complex the solvation to both groups (especially to the $^+NH_3$ group) may be sterically hindered by complex formation with 18-crown-6. The ΔG value (−3.0 kcal mol^{-1}) for the Trp–crown complex is a little less negative than those (−4.2 to −4.9 kcal mol^{-1}) of the tryptamine ammonium ion or its related compounds.[22] It is comparable to those of naphthylammonium ions (−2.3 to −4.4 kcal mol^{-1} at 300 K)[25] and phenanthrylammonium ions (−1.8 to −4.3 kcal mol^{-1} at 300 K).[27] These values are in the range −9.0 kcal mol^{-1} > ΔG > −2.9 kcal mol^{-1} observed for the complexes of *tert*-butylammonium salts with crown ethers.[33]

Internal Quenching of Tryptophan. Kinetic analyses of the excited singlet state of Trp with and without 0.5 M 18-crown-6 have been carried out in CH_3OH–H_2O (9:1) at various temperatures by means of the nanosecond time-resolved single photon counting method (Horiba NAES-1100). Typical results at 290 K (Figure 4) show (a) the observed fluorescence response function $I_0(t)$ for free Trp in the absence of 18-crown-6 and (b) the observed fluorescence response function $I(t)$ for the Trp–18-crown-6 complex monitored at 342 nm, together with the lamp function I_L, monitored at 280 nm. The decay features of $I(t)$ for free Trp show the double-exponential functions: τ_1 = 2.1 ns (97%) and τ_2 = 7.1 ns (3%) at 290 K within a 10% error limit. A subnanosecond component (e.g., 0.53 ns[9]) could not be observed in the present work. Even if it were present, the detection of a subnanosecond, minor component would be beyond the accuracy of the single-photon counter used. For the Trp–crown complex, the fluorescence function $I(t)$ shows single-exponential decay with a lifetime of τ_2^{max} = 7.3 ns at 290 K within a 10% error limit, which is very close to that (7.1 ns) of τ_2. The fluorescence lifetimes obtained are summarized in Table III. The difference in the fluorescence lifetimes between free Trp and the Trp–crown complex does not originate from the nature of their excited singlet states (^1L_a, ^1L_b), since no spectral change in absorption and emission is observed by complex formation of Trp with 18-crown-6 as described above. The difference in the fluorescence lifetime (or fluorescence in-

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Table III. Fluorescence Lifetimes (τ_1, τ_2, τ_2^{max}) and Rate Constants K_q for the Internal Quenching of Tryptophan at 290 K[a-c]

compd	solvent	[crown], M	lifetime, ns τ_1	lifetime, ns τ_2	lifetime, ns τ_2^{max}[d]	k_q, 10^8 s^{-1}
Trp	CH_3OH–H_2O (9:1)	0	2.1 (97)	7.1 (3)		3.4
		0.5			7.3 (100)	
TrpD	CH_3OD–D_2O (9:1)	0	3.2 (90)	7.6 (10)		1.8[e]
		0.5			7.7 (100)	

[a] Errors within 10%. [b] Parentheses denote percent of the decay components. [c] For details, see text. [d] [18-crown-6] = 0.5 M. [e] $k_q^D = k_\sigma^D(7.9 \times 10^7\ s^{-1}) + k_{CT}^D(1 \times 10^8\ s^{-1})$.

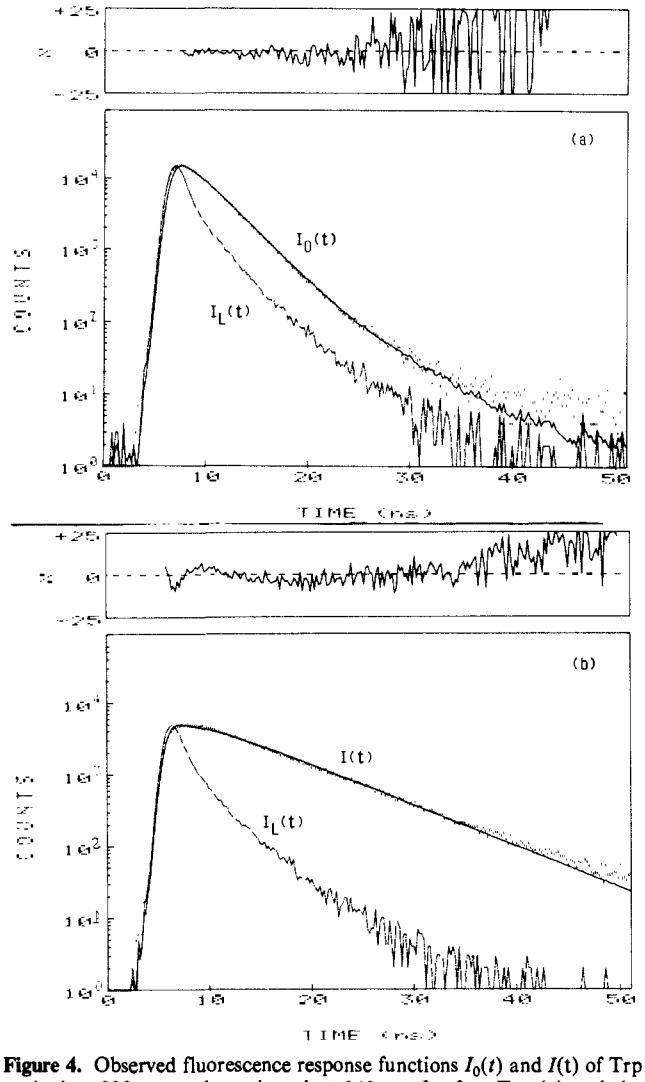
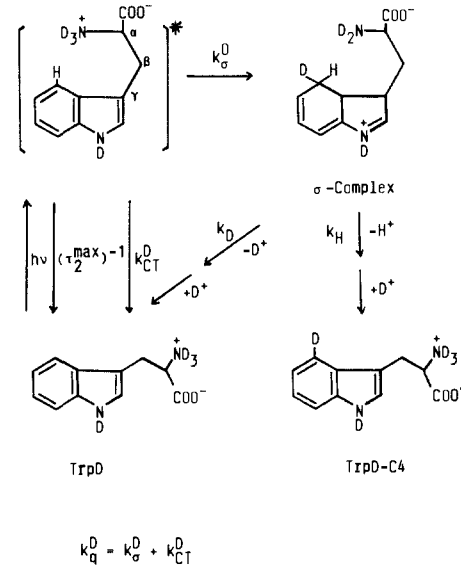


Figure 4. Observed fluorescence response functions $I_0(t)$ and $I(t)$ of Trp excited at 280 nm and monitored at 342 nm for free Trp (a) and the Trp–crown complex ([crown] = 0.5 M) (b), respectively, in CH_3OH–H_2O (9:1) at 290 K. The lamp functions monitored at 280 nm are shown as $I_L(t)$.

tensities) with and without 18-crown-6 is not attributable to the photoionization[20] or charge transfer to solvent (CTTS)[19] from the indole ring, since the quantum yield for the photoionization of Trp is known to be very small (ca. 0.001).[3] This difference is not due to the intermolecular quenching but is caused by intramolecular quenching. The ammonium group may interact intramolecularly with the excited indole moiety R*, resulting in the internal quenching. The complex formation of Trp with 18-crown-6 effectively prevents such interaction and leads to a relatively long lifetime (or relatively strong emission). This interpretation is exactly the same as that proposed in the cases of tryptamine and its related compounds.[22] Recently, James and Ware[34] reported that the fluorescence decay behavior of tryptophan-like molecules (indole-3-alkanoic acid) is explained in terms

(34) James, D. R.; Ware, W. R. *J. Phys. Chem.* **1985**, *89*, 5450.

Scheme I



of a dynamic interaction of the side chain with the solvated indole group during the excited lifetime.

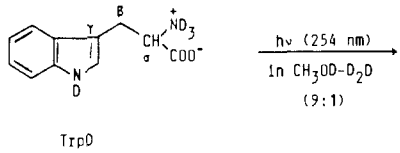
The double-exponential decay functions of Trp may arise from different ground-state C_α–C_β rotamers (the conformer model[9]) or from the modified conformers containing both C_α–C_β and C_β–C_γ rotamers in the ground state.[10] The results suggest that the rate for the internal rotation of Trp is comparable to the decay rate of Trp*. The relatively long lifetime τ_2 of Trp in the absence of 18-crown-6 may arise from a ground-state rotamer that gives no interaction between the ammonium group and the excited indole moiety R*. For tryptamine, with no carboxylate group in the side chain, the internal rotation leading to the quenching is not sterically restricted in the excited state.

Anyway, it is obvious that the internal quenching is caused by interaction between R* and $^+NH_3$ groups. The absolute value for the internal quenching rate (k_q) can be approximately estimated as shown in eq 5.[22] The k_q value in CH_3OH–H_2O (9:1) at 290 K is estimated as $3.4 \times 10^8\ s^{-1}$.

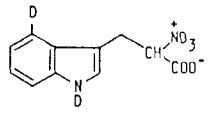
$$k_q = \tau_1^{-1} - (\tau_2^{max})^{-1} \quad (5)$$

Photochemical H–D Isotope-Exchange Reaction of Tryptophan. The photochemical H–D isotope-exchange reaction of Trp has been carried out in a CH_3OD–D_2O (9:1) mixture at 290 K. Recently, Saito et al.[15] have shown that the photochemical H–D isotope-exchange reaction of Trp occurs mainly at the C-4 position. In the present study, the kinetic H–D isotope-exchange reaction of TrpD at 254 nm at 290 K took place highly selectively at C-4 with the reaction quantum yield $\Phi_D = 0.16$ (±0.01) (eq 6).[35] On the other hand, the fluorescence of TrpD decayed with double-exponential functions of $\tau_1^D \simeq 3.2$ ns (90%) and $\tau_2^D = 7.6$ ns (10%) at 290 K. The fluorescence decay of the TrpD–18-crown-6 in CH_3OD–H_2O (9:1) at 290 K showed a single-exponential function with a lifetime of $(\tau_2^D)_{max} = 7.7$ ns (see Table III). Thus, the rate constant k_q^D for the internal quenching of TrpD in CH_3OD–D_2O (9:1) was approximately estimated as 1.8×10^8

(35) The conversion was about 10%. The amount of the H–D-exchanged product was proportional to the irradiation time.



TrpD



TrpD-C4 (6)

s^{-1} at 290 K from the equation $k_q^D \simeq (\tau_1^D)^{-1} - [\tau_2^D)_{max}^{-1}$. The experimental results can be accounted for by Scheme I, where k_σ^D denotes the rate constant for the electrophilic deuteriation at C-4 to produce the σ complex and k_{CT}^D represents the rate constant for the deactivation process through the intramolecular CT interaction between the excited indole R* (electron-donor) and the ammonium (electron-acceptor) groups. The deprotonation from the σ complex gives the H–D-exchanged product TrpD-C4, while the starting material TrpD is regenerated by deuteron loss from the σ complex. The ratio (k_H/k_D) of the rate constants for deprotonation vs deuteron loss is estimated to be 1.7 from an analogous reaction.[24] The reaction quantum yield Φ_R^D for the H–D-exchange reaction is, therefore, expressed as eq 7, where

$$\Phi_R^D = \frac{k_\sigma^D}{k_f + k_d + k_q^D} \frac{k_H}{k_H + k_D} = k_\sigma^D \tau_1^D k_H (k_H + k_D)^{-1} \quad (7)$$

values of Φ_R^D, τ_1^D $[=(k_f + k_d + k_q^D)^{-1}]$, and k_q^D are 0.16 and 3.2 ns and 1.8×10^8 s^{-1} at 290 K, respectively, and the value of $k_H(k_H + k_D)^{-1}$ is estimated as 0.63.[24] From eq 7 the k_σ^D value was obtained as 7.9×10^7 s^{-1}. These data are listed in Table III. It is noteworthy that the value of k_σ^D is less than half in comparison with that (1.8×10^8 s^{-1}) of k_q^D. The rate constant k_q^D for the internal quenching in CH_3OD–D_2O (9:1) consists of k_σ^D and k_{CT}^D (eq 8) as shown in Scheme I. Thus, the rate constant k_{CT}^D for

$$k_q^D = k_\sigma^D + k_{CT}^D \quad (8)$$

the internal quenching is estimated as 1.0×10^8 s^{-1}. Probably, the internal quenching with the rate constant of k_{CT}^D corresponds to the intramolecular CT quenching between the excited indole R* (electron donor) and the ammonium ion (electron acceptor). For tryptamine, the internal quenching is caused only by the intramolecular electrophilic protonation at the C-4 position of the excited indole moiety (R*).[22] It is known that the external proton-induced quenching of aromatic compounds such as methoxynaphthalenes occurs efficiently due to the electrophilic protonation at one of the carbon atoms of the aromatic ring, leading to the hydrogen exchange (or deuterium exchange).[23,24] The LUMO coefficients of indole nuclei calculated by the INDO SCF method are 0.414 (C-2), 0.502 (C-4), and 0.458 (C-7).[36]

(36) The LUMO coefficients were calculated by Dr. K. Yamaguchi, Osaka University, by using the INDO SCF method.

There are possibilities of the proton attack of the ammonium group at one of these three positions in the excited state. However, the Corey–Pauling–Kolton molecular model suggests that only the C-4 position can interact with the ammonium group in the excited state by rotation of the side chain, as already pointed out in the case of tryptamine.[22]

The experimental results suggest that the presence of the carboxylate group adjacent to the ammonium group of the side chain restricts considerably the internal rotation of the $^+NH_3$ side chain, resulting in a decrease of the rate for the internal quenching due to electrophilic protonation at the C-4 position of R*. This assumption may be supported by the fact that nonexponential decay of Trp fluorescence was actually observed, as already reported in several other system.[9,10,14,19,21] The decay features of Trp show that rotamer interconversion (rotational motion) is not so fast (it may be comparable to that of the fluorescence decay). For Trp the internal CT quenching occurs efficiently in addition to the quenching due to the electrophilic protonation at C-4. This implies that the excited-state Trp probably has a molecular conformation (presumably a face to face conformation between R* and $^+NH_3$ groups) favorable to the occurrence of the intramolecular CT quenching and that its conformation may be attained by rotation around C_α–C_β and C_β–C_γ axes of the alanyl side chain during the lifetime in the excited state. The charge-transfer quenching of Trp by the electrophilic side chain ($^+NH_3$ group) has been proposed by several other workers.[6-10] The electrophilicity of the side chain is increased by protonation to the amino group.[3] Since there is no electrophilicity for the carboxylate group, CT quenching of R* by the carboxylate group occurs very little.[12]

Summary

(1) The association constants K_g for the formation of the 1:1 tryptophan–18-crown-6 complex in CH_3OH–H_2O (9:1) at several temperatures are determined by the fluorimetric method, and the thermodynamic parameters are obtained.

(2) The fluorescence of the complex decays single exponentially with a lifetime of $\tau_2^{max} = 7.3$ ns in CH_3OH–H_2O (9:1), while the fluorescence decay of free tryptophan shows double-exponential functions with lifetimes of $\tau_1 = 2.1$ ns (97%) and $\tau_2 = 7.1$ ns (3%). As a result, complex formation increases markedly the fluorescence intensity of Trp.

(3) The experimental results clearly show that the quenching mechanism of Trp is not due to the external quenching, but to the internal quenching. The rate constant k_q for the internal quenching can be estimated from the equation $k_q = \tau_1^{-1} - (\tau_2^{max})^{-1}$.

(4) The photochemical H–D isotope-exchange reaction reveals that the internal quenching occurs significantly by both electrophilic protonation by the $^+NH_3$ group at the C-4 position of the excited indole moiety and by the charge-transfer interaction between the excited indole (electron donor) and the ammonium group (electron acceptor) of the side chain.

Registry No. Trp, 73-22-3; 1:1 Trp–(18-crown-6) complex, 112741-09-0; 18-crown-6, 17455-13-9.