Fluorescence as a Means for Kinetic Studies. III. Bimolecular Reaction of Fluorescent Reagent as Quenching Probes

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Synopsis. In order to study the rate of bimolecula reaction between 9-(3-aminopropyl)carbazole and dansyl chloride, the change of fluorescent intensity of dansyl group has been measured. It has been found that the rate of increase in fluorescent intensity of the dansyl group is equal to the rate of decrease in fluorescent intensity of the carbazolyl group during progress of this bimolecular reaction. Another *quenching probe* was also examined briefly.

Fluorescent probes are defined as fluorescent molecules whose spectral response (quantum yield, excitation, and emission) varies as a function of the physical and chemical environment with which the probe is associated.[1] In recent years the studies of bimolecular reaction rates in nonaqueous solution by using the fluorescent probe have been reported by many authors.[2-7] Especially, the fluorescent probe method is effective for the study on reaction rate of macromolecules because the rates of bimolecular reaction between polymers must be measured in a very dilute solution.[8]

Instrumental sensitivity for the detection of fluorescence emission is usually several orders of magnitude greater than that for absorption measurements. Thus the fluorescent probe method is the most effective for the study on rates of organic reaction in dilute solution. But there is a difficult problem on fluorescent probe method because the probes are restricted to special organic compounds. The most remarkable characteristics of fluorescent probe is the fact that these probes must have a fluorescence emission only after this reaction.

The reaction product between the one having energy donor group and the other having energy acceptor group shows the intramolecular energy transfer such as 9-[3-(5-dimethylamino-1-naphthylsulfonylamino)propyl]carbazole (DNSPC) shows the intramolecular singlet-singlet energy transfer between carbazolyl group (energy donor) and dansyl group (energy acceptor). So, it is contemplated that the decrease in fluorescent intensity based upon singlet-singlet energy transfer will be observed during progress of the bimolecular reaction between the compound having energy donor group and the other having energy acceptor group.

In this paper, the rates of bimolecular reaction between amino group and chlorosulfonyl or carbonyl chloride groups in nonaqueous solution have been measured by *quenching probe method.*

Experimental

Materials. 9-(2-Cyanoethyl)carbazole (CEC) was prepared in a similar way to that reported by other authors.[9]

9-(3-Aminopropyl)carbazole (APC) was prepared by the reduction of CEC by using lithium aluminium hydride r($LiAlH_4$).[10]

Anthraquinone-2-sulfonyl chloride (ASCl) was prepared in a similar way to that reported by other author.[11] The each product was identified by infrared and NMR spectra.

Measurements. Sample solution in a rectangular quartz cell with cap was set in a thermostat (60 °C), and the fluorescence spectra were measured by using JASCO FP-550 spectrofluorometer. Dotite-Luminasol-grade ethyl acetate was used as a solvent for fluorescent measurements.

Results and Discussion

The fluorescence spectra in the bimolecular reaction between 9-(3-aminopropyl)carbazole (APC) and 5-dimethylamino-1-naphthalenesulfonyl chloride (dansyl chloride, DNSCl) in ethyl acetate at 60 °C are shown in Fig. 1. The kinetic scheme of this bimolecular reaction is shown below.

$$\mathrm{CazCH_2CH_2CH_2NH_2 + DNSCl \longrightarrow CazCH_2CH_2CH_2NHSO_2DN + HCl} \quad (1)$$

In organic solvents, hydrogen chloride formed reacts immediately with amine.[12]

$$\mathrm{CazCH_2CH_2CH_2NH_2 + HCl \longrightarrow CazCH_2CH_2CH_2NH_3^+Cl^-} \quad (2)$$

The rate of this bimolecular reaction can be measured by the increase in fluorescent intensity of the dansyl group (499 nm) because the dansyl group shows a fluorescence emission only after this reaction. The rate constants for pseudo-first-order reaction under the concentrations of $[APC]=3.9\times10^{-5}$ M, 1.0×10^{-3} M, and 2.0×10^{-3} M, $[DNACl]=3.9\times10^{-5}$ M were measured in ethyl acetate at 60 °C by the increase in fluorescent intensity of the dansyl group. The plots of pseudo-first-order reaction show a straight line. The values of k_2 for second-order reaction are 0.059 s^{-1} M^{-1} ($[APC]=3.9\times10^{-5}$ M), 0.038 s^{-1} M^{-1} ($[APC]=1.0\times10^{-3}$ M), and 0.032 s^{-1} M^{-1} ($[APC]=2.0\times10^{-3}$ M). On the other hand, it has been supposed that the rate of increase in fluorescent intensity for the dansyl group is equal to the rate of increase in fluorescent intensity for the carbazolyl group during progress of bimolecular reaction in Fig. 1. DNSPC must show the intramolecular singlet-singlet energy transfer between carbazolyl group (energy donor : 81 kcal) and dansyl group (energy acceptor : 57 kcal). Consequently, the rate for this bimolecular reaction can be determined by the decrease in fluorescent intensity of the carbazolyl group, and the values of k_2 obtained by *quenching probe method* agree well with that by fluorescent probe method.

The value of k_2 for the bimolecular reaction between buthylamine and dansyl chloride was measured in ethyl acetate at 60 °C by the fluorescent probe method, and

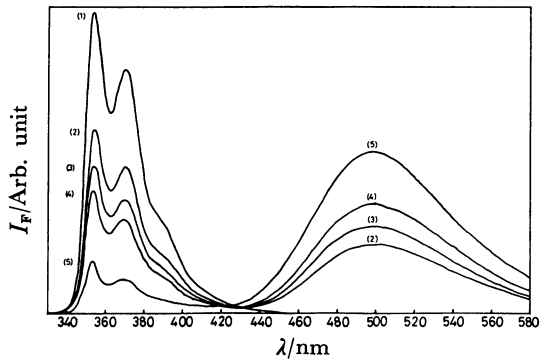

Fig. 1. Fluorescence spectra in the bimolecular reaction between 9-(3-aminopropyl)carbazole (APC) and dansyl chloride (DNSCl) in ethyl acetate. [APC]=[DNSCl]=3.9×10^{-5} M. Excitation wavelength: 350 nm. (1); 0 h (in the absence of DNSCl), (2); 51 h, (3); 94 h, (4); 143 h, (5); 598 h (at end point).

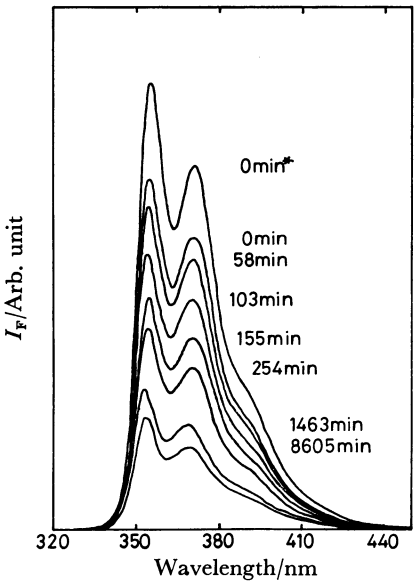

Fig. 2. Fluorescence spectra in the bimolecular reaction between 9-(3-aminopropyl)carbazole (APC) and antraquinone-2-sulfonyl chloride (ASCl) in ethyl acetate. [APC]=[ASCl]=3.9×10^{-5} M. Excitation wavelength: 350 nm. 0 min* is the condition in the absence of ASCl.

was 0.20 s^{-1} M^{-1}.[6] But the value of k_2 for the bimolecular reaction between APC and dansyl chloride under the above conditions was about 0.2—0.3 times as large as that for bimolecular reaction between butylamine and dansyl chloride. The bimolecular reaction between amino group and chlorosulfonyl group is well-known as the bimolecular nucleophilic substitution reaction (S_N2). The S_N2 displacement is less affected by electrometric effect (resonance, inductive, *etc.*) since there is relatively little charge on sulfur in the transition state.[13] Consequently, it seems reasonable to assume that the bimolecular reaction between APC and dansyl chloride is reduced by nonpolar effect of its own carbazolyl group.

The rates of bimolecular reactions between APC and ASCl or anthraquinone-2-carbonyl chloride (ACCl) in ethyl acetate at 60 °C can be measured by *quenching probe method* but not by fluorescent probe method. The kinetic schemes of those bimolecular reactions are below.

$$\mathrm{CazCH_2CH_2CH_2NH_2} + \mathrm{AQSO_2Cl} \longrightarrow \mathrm{CazCH_2CH_2CH_2NHSO_2AQ} \quad (3)$$

$$\mathrm{CazCH_2CH_2CH_2NH_2} + \mathrm{AQCOCl} \longrightarrow \mathrm{CazCH_2CH_2CH_2NHCOAQ} \quad (4)$$

The products formed by this bimolecular reaction shows the intramolecular singlet-singlet energy transfer between carbazolyl group (energy donor : 81 kcal) and anthraquinon-2-yl group (energy acceptor : 67 kcal). Moreover, anthraquinon-2-yl group does not fluoresce. The fluorescence spectra in the bimolecular reaction between APC and ASCl under the conditions of [APC]=[ASCl]=3.9×10^{-5} M in ethyl acetate at 60 °C are shown in Fig. 2. These values of k_2 obtained by plots for second-order reaction are 3.2 s^{-1} M^{-1}(between APC and ASCl) and 7.6 s^{-1} M^{-1} (between APC and ACCl). The values of k_2 for bimolecular reactions between APC and ASCl or ACCl in ethyl acetate at 60 °C are about 54 or 129 times as large as that for the bimolecular reaction between APC and dansyl chloride. The accelerating effect in the reaction with ASCl should be predictable from the distinction of electromeric effect on the sulfur atom in sulfonyl group between anthraquinon-2-yl group and dansyl group. These results are compatible with ones in other reaction between amino group and chlorosulfonyl or carbonyl chloried groups.

In conclusion, DNSPC formed by bimolecular reaction between APC and dansyl chloride shows the intramolecular singlet-singlet energy transfer between carbazolyl group and dansyl group. So, the decrease in fluorescent intensity based upon singlet-singlet energy transfer was observed during progress of the bimolecular reaction between the compound having energy donor group and the other having energy acceptor group. The rates of bimolecular reaction between APC and ASCl or ACCl could be measured by *quenching probe method.*

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