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# Kinetic and Spectrophotometric Studies of Rhodamine 6G Dimerization in Aqueous Solution

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The dimerization reaction of dye, Rhodamine 6G, in aqueous solution was studied spectrophotometrically and kinetically: the equilibrium constant and the rate constants were found to be $K=1.6\times10^3$ M$^{-1}$, $k_f=1.1\times10^9$ M$^{-1}$ s$^{-1}$, and $k_b=7.0\times10^5$ s$^{-1}$, respectively, at 15 °C. These rate constants, and those of other dyes are discussed. The effect of the ionic strength was also studied.

The aggregation of dye in solution has been of interest as a model system of the self-organization of sub-units into large structures of biological activity. The dimerization reaction of dye has been extensively investigated spectrophotometrically and kinetically. By the spectrophotometric experiments,[1–5] the equilibrium constant was obtained and the effect of additives has been studied. The kinetic experiments,[6–14] showed the rate constants to be very large. The rate constants of these dyes seem to be divided into two groups. Among them, Rhodamine B and 3B have been found to have relatively slow reaction rates, while the reaction of Rhodamine 6G was too fast to be observed by a conventional method.[12,13] Two purposes of the present work are to obtain the kinetic parameters of the dimerization of Rhodamine 6G and to discuss the difference in the rate constants of the two groups.

The increase of the ionic strength is known to promote the dimerization of dye.[3,15] Since the equilibrium constant is the ratio of the association and the dissociation rate constants, the ionic strength effect is also expected to be reflected in the rate constants. Another purpose is to investigate the effect of the ionic strength on this reaction.

## Experimental

Rhodamine 6G hydrochloride (Tokyo Kasei) was recrystallized three times from water and dried under vacuum at 110 °C for 12 h. Distilled and deionized water was used throughout the experiment. The ionic strength was adjusted by the addition of NaCl.

The equilibrium constant was determined spectrophotometrically with a Union Giken SM 401 spectrophotometer. The kinetic experiment was performed with a stimulated Raman laser temperature-jump apparatus; the details of the apparatus were described elsewhere.[9] The measurement was carried out at 15 °C.

## Results

Rhodamine 6G is known to aggregate in aqueous solution and this monomer-dimer equilibrium is established:

$$2\mathrm{M} \underset{k_b}{\overset{k_f}{\rightleftharpoons}} \mathrm{D} \tag{1}$$

where M is the monomer, D is the dimer, and $k_f$ and $k_b$ are the association and dissociation rate constants, respectively. The equilibrium constant $K$ is related to the molar extinction coefficient and the concentration of dye as follows:[15]

$$\{(\varepsilon_M-\varepsilon)/C_0\}^{1/2} = (2K/\Delta\varepsilon)^{1/2}\{\Delta\varepsilon-(\varepsilon_M-\varepsilon)\} \tag{2}$$

where

$\varepsilon_M$: the molar extinction coefficient of the monomer.

$\varepsilon$: the apparent molar extinction coefficient of dye.

$\Delta\varepsilon$: the difference between the molar extinction coefficient of the monomer and that of the dimer *per monomer unit*.

$C_0$: the initial concentration of dye.

According to Eq. 2, a plot of $\{(\varepsilon_M-\varepsilon)/C_0\}^{1/2}$ *vs.* $(\varepsilon_M-\varepsilon)$ will give a straight line with the intercept $(2K\Delta\varepsilon)^{1/2}$ on the ordinate and $\Delta\varepsilon$ on the abscissa. On this basis, $\varepsilon_M$ was evaluated to give the best straight line with the measured values of $\varepsilon$ and $C_0$. The plot is shown in Fig. 1. The equilibrium constant obtained, $K=1.6\times10^3$ M$^{-1}$ at 15 °C, seems to be satisfactory compared with the previously reported values.[4,5,13] Furthermore, the equilibrium constants at various ionic strengths were obtained in the same way; they are listed in Table 1. As shown in the table, with the increase of the ionic strength, the equilibrium constant increases, *i.e.*, the formation of dimer is promoted.

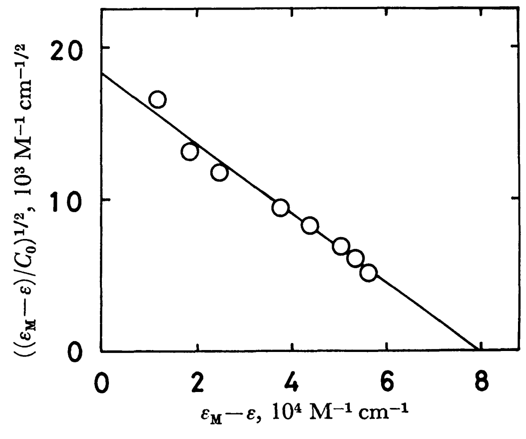

Fig. 1. Plots to determine the equilibrium constant $K$ for Rhodamine 6G dimerization at $\mu=0$ M, 15°C.

TABLE 1. THE RATE CONSTANTS AND THE EQUILIBRIUM CONSTANT FOR RHODAMINE 6G DIMERIZATION AT VARIOUS IONIC STRENGTHS (15 °C)

| Ionic strength (M) | $k_f$ ($10^9$ M$^{-1}$ s$^{-1}$) | $k_b$ ($10^5$ s$^{-1}$) | $K$ ($10^3$ M$^{-1}$) |
|---|---|---|---|
| 0.00 | 1.1 | 7.0 | 1.6 |
| 0.05 | 1.2 | 6.6 | 1.8 |
| 0.10 | 1.3 | 5.9 | 2.2 |
| 0.15 | 1.4 | 5.4 | 2.5 |

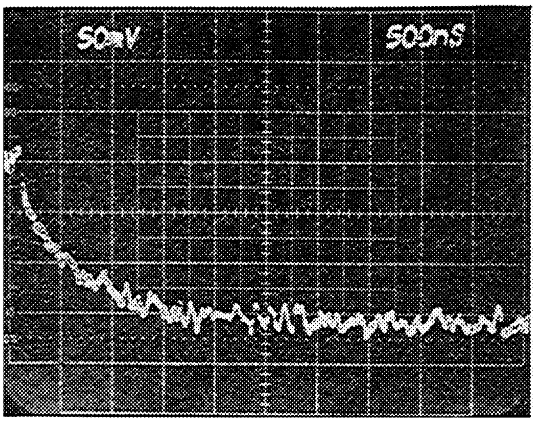

Fig. 2. Typical relaxation curve in $1.0\times10^{-3}$ M Rhodamine 6G at $\mu=0$ M, 15°C, wavelength 525 nm, sweep 500 ns/div.

The kinetic experiment of the dimerization reaction of dye was performed in the concentration range of $(0.5—4.0)\times10^{-3}$ M. The relaxation phenomenon was detected spectrophotometrically at the monomer band (525 nm). A typical relaxation curve is shown in Fig. 2. The relaxation was also observed at the dimer band (500 nm) in the same time range but the change of the relaxation was in the opposite direction; this relaxation curve was not used because of its small relaxation amplitude. Each relaxation curve was characterized by a single relaxation time. Relaxation times were reproducible to $\pm10\%$.

The relaxation time $\tau$ is related to the rate constants as follows:

$$\tau^{-1} = 4k_f\bar{C}_M + k_b \tag{3}$$

where $\bar{C}_M$ is the equilibrium concentration of the monomer, which is calculated from the equilibrium constant and the initial concentration. The plots of $\tau^{-1}$ against $\bar{C}_M$ are shown in Fig. 3, where every point of $\tau^{-1}$ is the mean value of at least 10 measurements. The good linear relationship indicates that the observed relaxation phenomenon is based on the monomer-dimer equilibrium of dye molecules. The value of $k_f$ was obtained from the slope of the straight line. Since the value of $k_b$ was small, it was calculated from $k_f$ and $K$ $(=k_f/k_b)$. The values of $k_f$ and $k_b$ obtained are listed in Table 1.

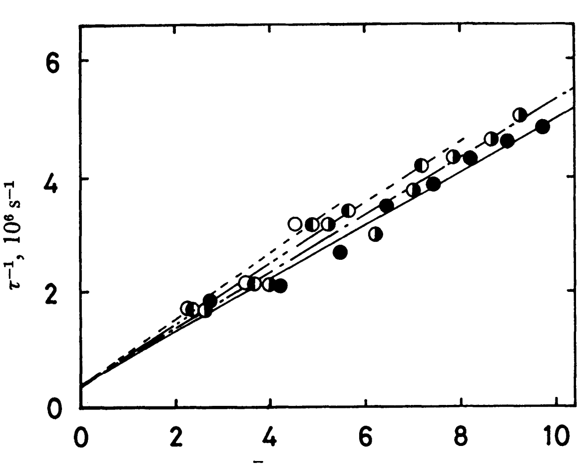

Fig. 3. Plots of $\tau^{-1}$ vs. $\bar{C}_M$ at various ionic strengths.
(●): $\mu=0$ M, (◑): $\mu=0.05$ M, (◐); $\mu=0.10$ M, (○): $\mu=0.15$ M.

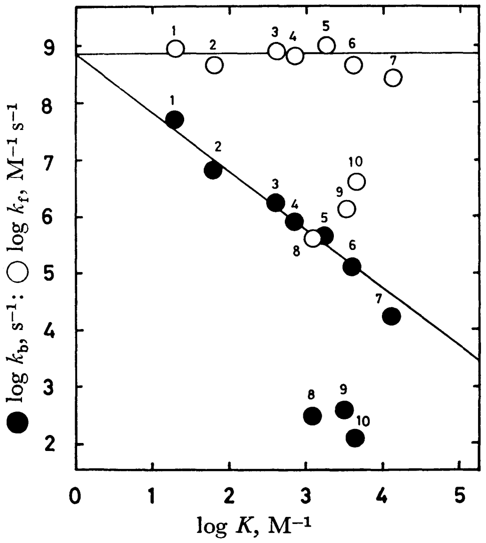

Fig. 4. Plots of log $k_f$ and log $k_b$ vs. log $K$ for various dyes.
1: Dimethyladenine (5), 2: Ethidium Bromide (6), 3: Proflavine (7), 4: Thionine (8), 5: Rhodamine 6G (this work), 6: Methylene Blue (10), 7: Acridine Orange (11), 8: Rhodamine B (12), 9: Rhodamine 3B (13), 10: Congo Red (14).
(The number in parenthesis means the reference number).

In order to compare the rate constants of dimerization of Rhodamine 6G with those of other dyes, log $k_f$ and log $k_b$ were plotted against log $K$ in Fig. 4. From the kinetic standpoint of view, dyes in this figure seem to be divided into two groups. In group I, the values of $k_f$ fall in the same order of magnitude and are close to that of the diffusion-controlled rate constant, and the linear relationship between log $k_b$ and log $K$ is recognized. On the other hand, in group II, the values of $k_f$ and $k_b$ are a few orders of magnitude smaller than the corresponding values in group I.

The effect of the ionic strength $\mu$ on the rate constants was studied under the conditions of $\mu=0.05$, 0.10, 0.15 M. At $\mu=0.15$ M, the measurement was limited to low concentrations since the dye precipitated at higher concentrations. The plots of $\tau^{-1}$ vs. $\bar{C}_M$ at different $\mu$ are also shown in Fig. 3. The rate constants obtained are listed in Table 1. As shown in the table, the increase of $\mu$ induces an increase of $K$, a small increase of $k_f$, and a small decrease of $k_b$.

## Discussion

The dimerization reaction 1 may be divided into the following two processes:

$$\mathrm{M} + \mathrm{M} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (\mathrm{M}, \mathrm{M}) \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{D} \tag{4}$$

where the first process is the diffusion-controlled reaction to form the encounter complex (M,M) and the second process is the intramolecular reaction to form the stable dimer D. Using the steady-state assumption for the encounter complex, the association and the dissociation rate constants are expressed as follows:

$$k_f = \frac{k_1k_2}{k_{-1}+k_2} \tag{5}$$

$$k_b = \frac{k_{-1}k_{-2}}{k_{-1}+k_2} \tag{6}$$

The diffusion-controlled rate constants $k_1$ and $k_{-1}$ can be theoretically estimated to be $k_1=1.8\times10^9$ $M^{-1}$ $s^{-1}$ and $k_{-1}=3.0\times10^9$ $s^{-1}$.[16] These values are in the same order of magnitude as those of other dyes; the difference of dyes is not reflected in the first step of Reaction 4. Inserting the values of $k_1$, $k_{-1}$, $k_f$, and $k_b$ into Eqs. 5 and 6, $k_2$ and $k_{-2}$ were calculated to be $4.7\times10^9$ $s^{-1}$ and $1.8\times10^6$ $s^{-1}$, respectively. Since the value of $k_2$ is very large and is nearly equal to $k_1$ and $k_{-1}$, the activation energy should be as small as that of the diffusion-controlled process. In the same way, the rate constants $k_1$, $k_{-1}$, and $k_2$ of other dyes in group I fall in the order of $10^9$. Accordingly the difference in the equilibrium constant of the dyes in group I, including Rhodamine 6G, is attributed to that of $k_{-2}$.

Schelly *et al.*[12,13] have investigated kinetically the dimerization reaction of Rhodamine dyes (B, 3B, and 6G) by the stopped-flow method; the reaction of Rhodamine 6G, however, was too fast to be observed. With use of the reported values of $k_f$ and $k_b$, the values for $k_2$ and $k_{-2}$ of both Rhodamine B and 3B were estimated to be of the order of $10^6$ and $10^{2.5}$, respectively. These rate constants are much smaller than the corresponding values of Rhodamine 6G, while the equilibrium constants of the dimerization of Rhodamine dyes are of the same order. These results can also be interpreted from the standpoint of free energy: the activation free energy of Rhodamine B and 3B is much higher than that of Rhodamine 6G, though the free energy differences are all of the same order (−4.1—−4.7 kcal/mol); the free energy diagram is schematically shown in Fig. 5. Taking into account the structure of these dyes, we see that the existence of two methyl groups in the xanthene plane of Rhodamine 6G does not affect the free energy difference, but does affect the activation free energy between the encounter complex and the stable dimer states. The fact that Rhodamine 6G reacts through a different pathway from Rhodamine B may also be suggested by the $\mu$ dependence of the rate constants: the $\mu$ dependence of the rate constant of Rhodamine B has been reported to be very large,[12] while that of Rhodamine 6G was found to be small.

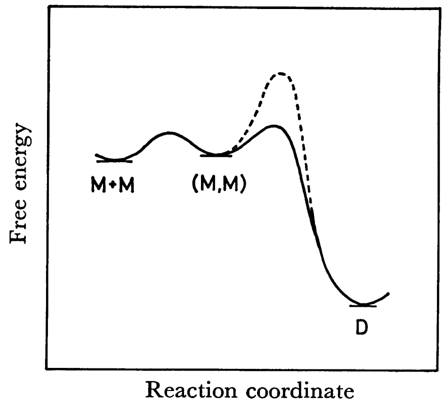

Fig. 5. Schematic diagram of the free energy of Rhodamine dyes.
(------): Rhodamine B and 3B, (——): Rhodamine 6G.

The ionic strength dependency of $k_f$ and $k_b$ given in Table 1 is realized in relation to Reaction 4. Since the dimerization is the reaction between the positive charges, the increase of the counter ion in solution decreases the electrostatic repulsion between dyes and promotes the dimerization, *i.e.*, $k_1$ increases with the increase of $\mu$, while $k_{-1}$ is unaffected.[18] Since the second step of Reaction 4 is the intramolecular process, the dependence of $k_2$ on $\mu$ may be small. Accordingly, with Eq. 5, the increase of $k_f$ with the increase of $\mu$ is interpreted by that of $k_1$. From the above considerations on the rate constants ($k_{-1}$ and $k_2$) with Eq. 6, the decrease of $k_b$ with the increase of $\mu$ is ascribed to that of $k_{-2}$. The decrease of $k_{-2}$ with the increase of $\mu$ may be understood by the contribution of the counter ion $Cl^-$ to the stabilization of the dimer.

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