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Kinetic Studies of the Dimerization Reaction of Thionine by the Laser Raman Temperature-jump Method

Wataru INAOKA, Shoji HARADA, and Tatsuya YASUNAGA
Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Hiroshima 730

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Kinetic studies of the dimerization reaction of thionine in an aqueous solution have been performed by the laser temperature-jump method with the use of the stimulated Raman effect in high-pressure H_2 gas. From the concentration dependence of the relaxation time, the formation and dissociation rate constants of the thionine dimer have been obtained. The data have been confirmed by the equilibrium constant, independently obtained spectrophotometrically. The formation rate constant of the dimer was very large, and the process was revealed to be a diffusion-controlled reaction, similar to those with other dyes.

The aggregation of a dye in solution is interesting not only in itself but also as a model system of molecular aggregation in the biochemical field. Many spectrophotometric[1–5)] and polarographic[6)] investigations have been performed. The kinetic investigations have, however, been limited in number because of their rapid aggregation rates and other experimental restrictions. The laser-heating temperature-jump method has a decisive advantage over the usual Joule-heating one for kinetic studies of the dye aggregation; it enables the measurement of a very rapid reaction with a time constant in even the nsec region and needs no addition of salts, which is undesirable for studies of the dye aggregation in solution. The recently developed method of the laser-heating T-jump with the use of a stimulated Raman effect in liquid N_2[7)] or high-pressure H_2 gas[8)] has much improved the applicability. In the present study, the laser T-jump method with a stimulated Raman effect in H_2 gas was applied to kinetic studies of the aggregation reaction of thionine in an aqueous solution.

Experimental

Materials. Thionine hydrochloride was purchased from Wako Pure Chemical Industries, Ltd. It was recrystallized from water and then dried at 105 °C for 12 h. The purity of the dye was confirmed by means of CHN gravimetric analysis. Distilled and deionized water was used throughout. The pH of the solution was controlled to 3.5 by the addition of nitric acid to prevent the dye decomposition.

Spectrophotometric Measurement. The equilibrium constant of the monomer-dimer reaction of thionine was determined spectrophotometrically with a Union Giken SM-401 spectrophotometer. The measurement was performed at 25 °C.

Laser Raman Temperature-jump Apparatus. The apparatus used in this study was a modification of that described by Ameen.[8)] A schematic diagram of our apparatus is shown in Fig. 1. The giant pulse laser (Japan Electron Optics Laboratory Co., Ltd. Type JLS-G-3) operates in the Q-switched mode with a rotating prism. The neodymium-doped glass rod (100 mm long, 12.5 mm diam.) delivers a maximum energy of 3 J in 25 ns at a wavelength of 1060 nm. The laser beam was focused into the Raman cell by means of a $f=50$ cm quartz lens. The Raman cell consists of a stainless steel tube (100 cm long, 20 mm i.d.) with 15 mm quartz windows at the ends and filled with H_2 gas of about 80 atm. Several kinds of lights with different wavelengths are emitted. To select only the 1890 nm light, a Si-coated filter (transmittance of 1060 nm, 8.5%; 1890 nm, 93%) was used, and then the light was again focused into the sample cell by means of a $f=20$ cm quartz lens. The sample cell is made of two glass plates, with a spacer between them. Several spacers 0.1—0.5 mm thick were used. In order to prevent cavitation, a pressure of a few atm was exerted on the sample solution. The Raman cell, filter, and lens were completely covered to prevent the visible light produced by the Raman scattering from entering the photomultiplier. The laser axis was aligned by means of a conventional laser beam. The relaxation effect was monitored photometrically. The light from a 500-W xenon lamp was monochronized and focused into the sample cell. A shutter was used to prevent the decoloration of the sample. The detection axis was aligned so as to have an angle of about 30 degrees to the laser axis, -thus having a long path length in the solution. As a photomultiplier tube, 931A (Hamamatsu TV Co., Ltd.) was employed. The output signal was amplified and led to the emitter follower, the filter, and the oscilloscope. The apparatus was calibrated by means of the phenolred (1 mM)-ammonia (2.3 M) system at pH 7.9. This system is known to have a large enthalpy change and a very short relaxation

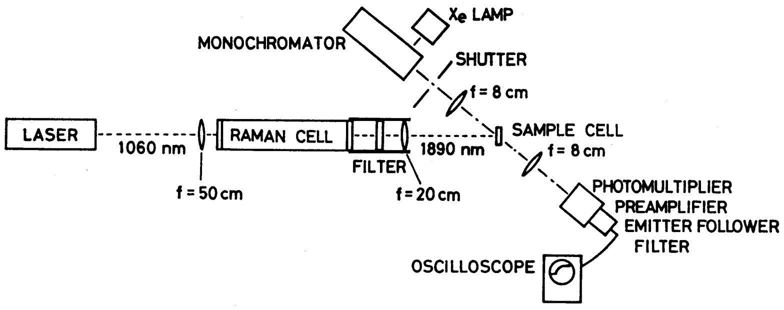

Fig. 1. Schematic diagram of the laser temperature-jump apparatus with use of the stimulated Raman effect in H_2 gas.

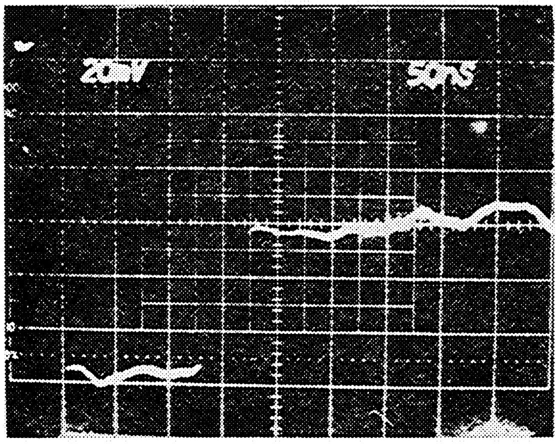

Fig. 2. Spectrum in the phenolred (1 mM)-ammonia (2.3 M) system at pH 7.9, 25 °C; sweep 50 ns/div.

time of about 10 ns.[9] The spectrum is shown in Fig. 2. In the case of a heating volume of 5 μl, the magnitudes of the temperature-jump and the heating-time constant were determined to be 3 °C and 50 ns respectively.

Results and Discussion

Thionine is known to aggregate in an aqueous solution and a monomer-dimer equilibrium is established:

$$2\mathrm{T} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{T}_2, \quad (1)$$

where k_1 and k_{-1} are the formation and dissociation rate constants respectively. The equilibrium constant, K, of this system is expressed by $K=[\mathrm{T}_2]/[\mathrm{T}]^2=k_1/k_{-1}$, where $[\mathrm{T}]$ and $[\mathrm{T}_2]$ denote the monomer and dimer concentrations respectively. In order to obtain the K value, spectrophotometric studies were performed with the following equation:[10]

$$\left(\frac{\varepsilon_M-\varepsilon}{C_0}\right)^{1/2} = \left(\frac{2K}{\Delta\varepsilon}\right)^{1/2}\{\Delta\varepsilon-(\varepsilon_M-\varepsilon)\}, \quad (2)$$

where ε_M: the molar extinction coefficient of the monomer.
ε: the apparent molar extinction coefficient.
$\Delta\varepsilon$: the difference between the molar extinction coefficient of the monomer and that of the dimer.
C_0: the initial concentration of the 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, we evaluated ε_M as the best choice to obtain a straight line with the measured values of ε and C_0. The value of K was obtained as 750 M^{-1} at 25 °C. In comparison with the previously reported values of this dye,[2,11,12] the present result seems to be satisfactory.

The kinetic measurements were carried out at 25 °C. The concentration of the dye was ranged from 0.2 to 2.4 mM. The concentration change was monitored at the monomer band (λ=597 nm). The relaxation phenomenon was observed over all the concentration range and was characterized by a single relaxation time. The concentration dependence of the relaxation time, τ, is expressed as follows:

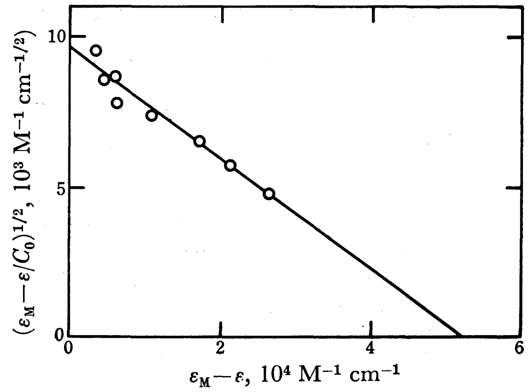

Fig. 3. Plots to determine the equilibrium constant K of dimerization at pH 3.5, 25 °C.

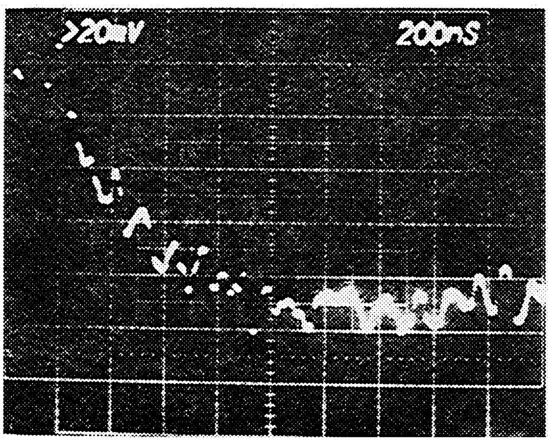

Fig. 4. Typical relaxation spectrum in 2 mM thionine at pH 3.5, 25 °C, wavelength 597 nm; sweep 200 ns/div.

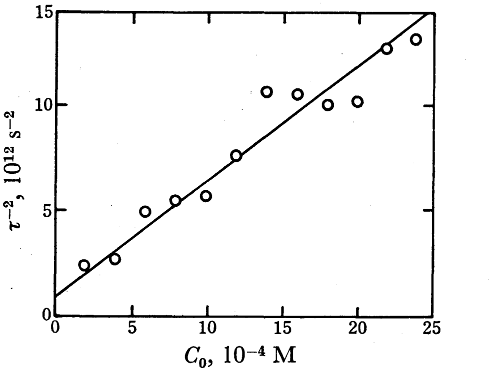

Fig. 5. Plots of τ^{-2} *vs.* C_0 at pH 3.5, 25 °C.

$$\tau^{-1} = 4k_1[\mathrm{T}] + k_{-1}. \quad (3)$$

This equation can be arranged more conveniently with the initial concentration of the dye:

$$\tau^{-2} = k_{-1}^2 + 8k_1k_{-1}C_0. \quad (4)$$

The experimentally obtained values of τ^{-2} are plotted against C_0 in Fig. 5. Each value is the average of at least ten runs. The linearity of these plots indicates that the observed relaxation effect is based on the monomer-dimer reaction of the dye. The rate constants were evaluated to be $k_1=7.1\times10^8$ M^{-1} s^{-1} and $k_{-1}=9.4\times10^5$

Table 1. Summary of the rate and equilibrium constants for dimerization reactions at 25 °C

Dye	k_1 (10^8, $M^{-1}\,s^{-1}$)	k_{-1} (10^6, s^{-1})	K (M^{-1})	Ref.
Ethidium bromide	4.6	6.7	69	13
Proflavine	7.9	2.0	395	14
Acridine orange	2.7	0.018	15000	15
N^6,N^9-Dimethyladenine	9.3	50	19	16
Thionine	7.1	0.94	750	This work

s^{-1} from the slope and the intercept respectively of this straight line. The resultant equilibrium constant given by the ratio of these values is in good agreement with that derived spectrophotometrically. The rate constants were then compared with those obtained for other dyes; they are listed in Table 1. The rate and equilibrium constants of thionine are very analogous to those of proflavine. This fact is confirmed by their similar structures. As can be said for other dyes, the value of k_1 of thionine is very large and close to that of the diffusion-controlled association reaction.[17)] If the dimerization process of the dye is a diffusion-controlled reaction, the value of k_1 will decrease in the case of a bulky dye molecule since the diffusion coefficient depends on the volume of the molecule. These tendencies in Table 1 show that the dye aggregation process is a diffusion-controlled reaction. In contrast to the small difference between the k_1 values, the values of k_{-1} differ remarkably; *i.e.*, the difference in the equilibrium constants is directly reflected in the values of k_{-1}. The good linear relationship between log k_{-1} and log K pointed out by Turner *et al.*[13)] is here supported.

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