Studies of the Chemiluminescence of Several Xanthene Dyes. VI. A Kinetic Analysis of a Blue-fluorescent Product in the Chemiluminescent Reaction of Eosin Y with Hydrogen Peroxide

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In the present study, we found that the absorbance at 395 nm of the chemiluminescent reaction system of eosin Y with hydrogen peroxide initially increased to a maximum, and then gradually decreased. From the finding that the absorption spectrum of a chromatographically-isolated blue-fluorescent product (M_E) showed a peak at the same wavelength (395 nm), we could confirm that the increment of the absorbance at 395 nm (Δ_{395}) was mainly due to the generation of M_E; we then extended the investigation of the mechanism for the chemiluminescent reaction by a kinetic analysis of M_E. The observed value of Δ_{395} could be approximately calculated by the expression of $[M_E]$, which was derived from a kinetic scheme proposed in the previous paper.[1] The ratio of the generation rates of M_E in different media, which was calculated from the observed values of Δ_{395}, was in good agreement with that estimated from the curves of the chemiluminescence emission intensity *versus* the time measured in the media. These results gave additional evidence in support of the view that the blue-fluorescent species is the primary excited product in the reaction.

In the previous paper,[1] Kamiya and Kato showed that the chemiluminescence emission (CL-emission) in the reaction of such a xanthene dye as eosin Y with hydrogen peroxide in an alkaline solution was composed of two emission components: a greenish-yellow emission whose spectral distribution was similar to that of the fluorescence emission (FL-emission) of the dye, and a blue emission similar to the fluorescence band which appeared during the course of the reaction. The observed characteristics of the emission components indicated that the generation of an excited species which fluoresces blue light, and an energy-transfer process from the exicted product to an unoxidized dye were likely to be part of the mechanism for the chemiluminescent reaction.

In the present study, we found that the absorbance at 395 nm of the reaction system initially increased to a maximum, and then gradually decreased. From the finding that the absorption spectrum of a chromatographically-isolated blue-fluorescent product showed a peak at the same wavelength (395 nm), we could confirm that the increment of the absorbance at 395 nm (Δ_{395}) was mainly due to the generation of the blue-fluorescent product (M_E); we then extended the investigation of the mechanism for the chemiluminescent reaction by a kinetic analysis of M_E. The details of the experimental results and a discussion based on the results will be presented in this paper.

Experimental

The luminescent reaction was performed by adding 0.5 ml of a 20% aqueous solution of hydrogen peroxide to a mixture of 1 ml of a 0.3 M aqueous solution of sodium hydroxide and 3 ml of a 1.3×10^{-3} M eosin Y solution in 50% dimethylsulfoxide, in 50% ethanol, or in water, in a quartz cell ($10\times10\times45$ mm) at 40 °C.

The FL-emission spectra were measured on a Hitachi MPF-2A-type recording fluorescence spectrophotometer. With the exciting source turned off, the intensities of the CL-emission were measured on the same apparatus equipped with a thermostatic cell assembly. The absorption spectrum and the changes in the absorbances at 395 nm and 517 nm with the reaction time were measured on a Hitachi 124-type recording spectrophotometer. The temperature of the reaction system was maintained at 40 °C in a Komatsu SPR-D Coolnics cell holder.

The separation of the blue-fluorescent product from the reaction mixture could be performed by thin-layer chromatography (Silica Gel G on a glass plate; chloroform–acetic acid, 1 : 1, or acetone–benzene–acetic acid, 2 : 7 : 1, for development), as well as by paper chromatography (Toyo Filter Paper No. 50; 25% aqueous ethanol solution–5% aqueous ammonia solution, 1 : 1, for development). A part of the blue-fluorescent spot on the plate was scraped off, and the blue-fluorescent product was extracted with ethanol.

The spent reaction mixture was neutralized with dilute hydrochloric acid and evaporated to dryness; then the main product of the reaction was extracted with ethanol. The identification of the main product with an authentic sample of phthalic acid was performed on a Yanagimoto GCG-550-type gas chromatograph, in which nitrogen gas was used as carrier gas, and on a Hitachi EPI-G-type infrared spectrophotometer (KBr-disk method).

Results and Discussion

Figure 1 shows the change in the absorption spectrum of the chemiluminescent reaction system of eosin Y with hydrogen peroxide (50% dimethyl sulfoxide system) in the region of 330—470 nm.[2] This figure indicates that the absorbance at 395 nm initially increases to a maximum, and then gradually decreases. Figure 2 illustrates the curves of the absorbance at 395 nm *versus* the reaction time (Δ_{395}–t curves) for the reaction in three media: a 50% aqueous solution of dimethyl sulfoxide (50% DMSO), a 50% aqueous ethanol solution (50% EtOH), and water (H_2O). The results clearly demonstrate that the Δ_{395}–t curves are strongly characterized by the media; that is, Δ_{395} is remarkable in 50% DMSO, prolonged but not so remarkable in 50% EtOH, and slight in H_2O. It was found that the absorption spectrum of an ethanol solution of the isolated blue-fluorescent product showed a peak at 395 nm, as is illustrated in Fig. 3. This result strongly suggests that the change in the absorbance at

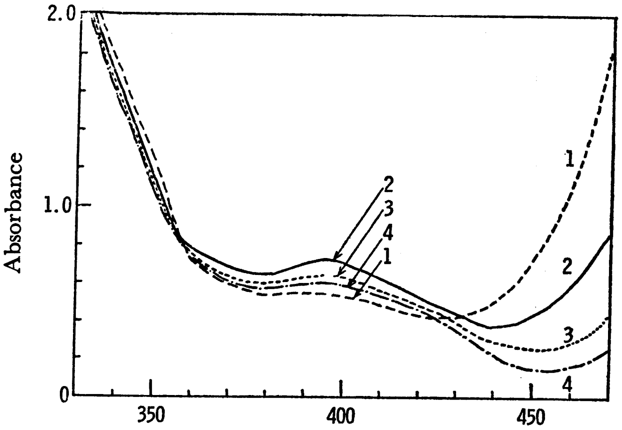

Fig. 1. The change of the absorption spectrum of the chemiluminescent reaction system of eosin Y with hydrogen peroxide (50% DMSO) in the region of 330—470 nm. 1: before adding H_2O_2, 2→4: after adding H_2O_2.

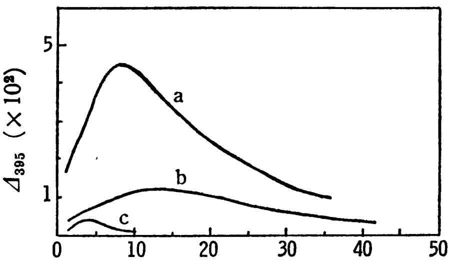

Fig. 2. Δ_{395}–t curves on the chemiluminescent reaction of eosin Y with hydrogen peroxide in three different media. a: in 50% DMSO, b: in 50% EtOH, c: in H_2O (40 °C).

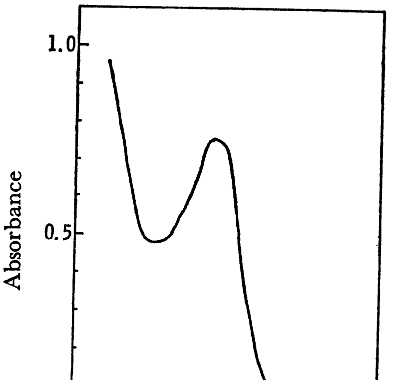

Fig. 3. Absorption spectrum of a ethanol solution of isolated blue-fluorescent product.

395 nm is mainly due to the generation of the blue-fluorescent product.

The overall reaction in the chemiluminescent system seems to be complicated, since several spots of minor unknown reaction products other than the main product (phthalic acid) and the blue-fluorescent product were separated by chromatography. However, without regard to the details, it has been found that the experimental results in the previous studies are consistent with the following kinetic scheme.[1,3)]

Radical generation from the decomposition of hydrogen peroxide in an alkaline solution:

$$H_2O_2 \longrightarrow R^{\cdot}\ (O_2H^{\cdot} \text{ or } OH^{\cdot})$$

The generation of an excited (blue-fluorescent) species (M_E) by the decomposition of a short-lived energy-rich intermediate (M) which will be produced by the reaction of eosin Y (E) with $R\cdot$:

$$E + R^{\cdot} \longrightarrow M \longrightarrow M_E^* \quad \text{(reaction rate } k_1') \quad \text{(i)}$$

An energy-transfer process:

$$M_E^* + E \longrightarrow M_E + E^* \quad (k_2) \quad \text{(ii)}$$

Blue FL-emission from M_E^*:

$$M_E^* \longrightarrow M_E + h\nu_1 \quad (k_3) \quad \text{(iii)}$$

Greenish-yellow FL-emission from E^*:

$$E^* \longrightarrow E + h\nu_2 \quad (k_4) \quad \text{(iv)}$$

Radiationless deactivation of E^*:

$$E^* \longrightarrow E + \text{heat} \quad (k_4') \quad \text{(iv')}$$

Self-quenching:

$$E^* + E \longrightarrow E + E \quad (k_5) \quad \text{(v)}$$

Since the Δ_{395}–t curve decreased in the course of the reaction,[4)] the decomposition step of M_E may be taken into account:

$$M_E + R^{\cdot} \longrightarrow Q \quad (k_6) \quad \text{(vi)}$$

The quantity of blue-fluorescent species (M_E) produced must be small because of the low quantum yield of this chemiluminescent reaction.[5)] Product Q, therefore, seems not to be the main reaction product (phthalic acid, P), since the yield of P (based on eosin Y consumed) was about 40%; therefore a further generation step of P which competes with reaction (i) must be present:

$$E + R^{\cdot} \rightarrow\rightarrow P \quad (k_7') \quad \text{(vii)}$$

According to the reaction scheme, the intensity of the greenish-yellow CL-emission ($h\nu_2$, I_E) is given by:

$$I_E = k_1'[R^{\cdot}]k_2k_4[E]^2/(k_2[E]+k_3)(k_4+k_4'+k_5[E]) \quad (1)$$

where [E] is the concentration of E at time t.

Since the intensity of blue CL-emission ($h\nu_1$), which is given by $k_1'[R\cdot][E]k_3/(k_2[E]+k_3)$, and the fluorescence efficiency of eosin Y did not vary much with the change in [E] in the systems, assuming that $k_2[E] \gg k_3$ and $k_4+k_4' \gg k_5[E]$, and further assuming that the reactions of (i) and (vii) obey the pseudo-first order reaction in view of the previous result, that $[R\cdot]$ did not vary during the reaction provided $[H_2O_2]$ was high (excess), we can write:

$$I_E = \{k_1k_4/(k_4+k_4')\}[E]_0 \exp\{-(k_1+k_7)t\} \quad (2)$$

where $k_1 = k_1'[R\cdot]$, $k_7 = k_7'[R\cdot]$, and where $[E]_0$ is the initial concentration of E.

The straight lines for the plots of log I_E (the lograithm of the CL-emission peak at 560 nm) *versus* the reaction time shown in Fig. 4 indicate that Eq. (2) is acceptable, and from the lines the values of k_1+k_7 in

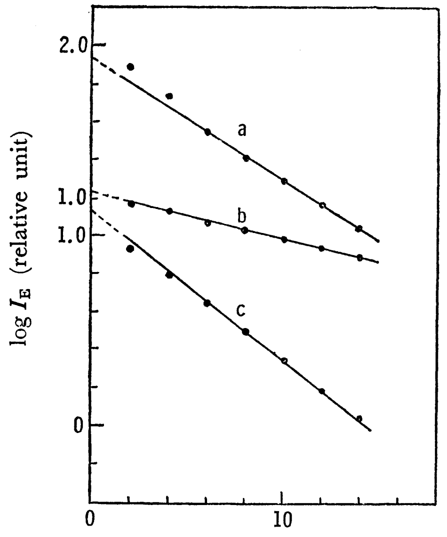

Fig. 4. Log plots of the relative intensities of emission peak (I_E) at 560 nm *versus* reaction time for the chemiluminescent reaction of eosin Y in 50% DMSO (a), 50% EtOH (b), and H_2O (c) at 40 °C.

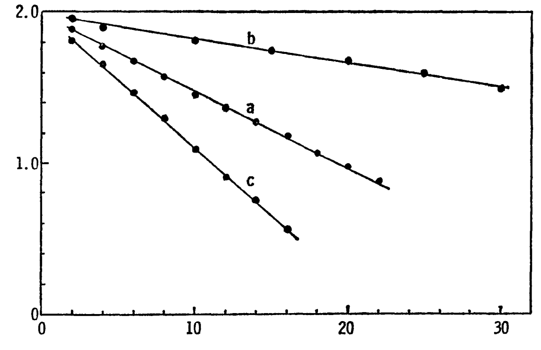

Fig. 5. Log plots of the absorbance at 517 nm of eosin Y (decomposed during the course of the chemiluminescent reaction) *versus* reaction time in 50% DMSO (a), 50% EtOH (b), and H_2O (c) at 40 °C.

50% DMSO, 50% EtOH, and H_2O are estimated to be 0.14, 0.05, and 0.19 min^{-1} respectively under the experimental conditions employed in the study. The same values of k_1+k_7 could be found from the lines for the log plots of the absorbance at 517 nm (the absorption peak of eosin Y) *versus* the reaction time (see Fig. 5).

The decomposition path of E being summarized by the following competitive and consecutive reaction diagram:

$$\mathrm{E} + \mathrm{R}^{\cdot} \begin{cases} \xrightarrow{k_1'} \mathrm{M_E^*} \xrightarrow{\text{(fast)}} \mathrm{M_E} \xrightarrow{k_6'} \mathrm{Q} \\ \xrightarrow{k_7'} \longrightarrow \mathrm{P} \end{cases}$$

the concentration of the blue-fluorescent product ($[M_E]$) at time t can be given by:

$$[\mathrm{M_E}] = \{k_1[\mathrm{E}]_0/(k_1+k_7-k_6)\}[\exp(-k_6t) - \exp\{-(k_1+k_7)t\}] \quad (3)$$

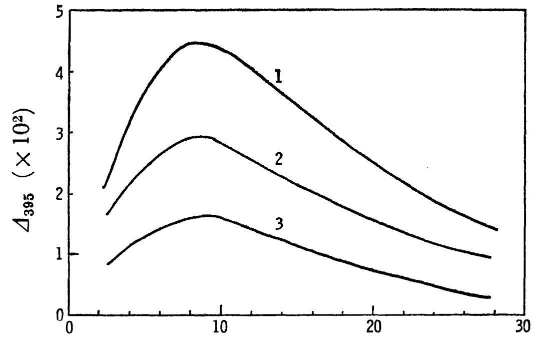

Fig. 6. Δ_{395}-t curves measured in 50% DMSO systems of different initial concentrations of eosin Y. 1: 8.7 ×10^{-4} M, 2: 5.8×10^{-4} M., 3: 4.3×10^{-4} M (40 °C).

where $k_6'[\mathrm{R}\cdot]$ is put to k_6 by assuming that Reaction (vi) also obeys the pseudo-first order reaction.

Figure 6 shows Δ_{395}-t curves measured in 50% DMSO systems of different initial concentrations of eosin Y($[\mathrm{E}]_0$). The results indicate that the value of Δ_{395} is strongly proportional to $[\mathrm{E}]_0$, and so is proportional to $[\mathrm{M_E}]$, if Eq. (3) is acceptable. From this argument, and recalling the results shown in Fig. 3, even though Δ_{395} may be changed a little by the decomposition of eosin Y and the generation of other minor, unknown reaction products, it seems reasonable to assume that:

$$\Delta_{395} \fallingdotseq \varepsilon_{395}[\mathrm{M_E}] \quad (4)$$

where ε_{395} is the molar extinction coefficient of $\mathrm{M_E}$ at 395 nm. From Eq. (4), we can write:

$$\Delta_{395} = \{k_1\varepsilon_{395}[\mathrm{E}]_0/(k_1+k_7-k_6)\}[\exp(-k_6t) - \exp\{-(k_1+k_7)t\}] \quad (5)$$

From Eqs. (3) and (4), putting t_m as the time required the reach to maximum of Δ_{395}, we can derive the following equation:

$$k_6\exp(-k_6t_\mathrm{m}) = (k_1+k_7)\exp\{-(k_1+k_7)t_\mathrm{m}\} \quad (6)$$

since $d[\Delta_{395}]/dt=0$; therefore $d[\mathrm{M_E}]/dt=0$ at $t=t_\mathrm{m}$. From the curves shown in Fig. 2, t_m being found to be 8, 12, and 2 min in 50% DMSO, 50% EtOH, and H_2O respectively, we can calculate the values of k_6 to be 0.10, 0.13, and 1.1 min^{-1} in 50% DMSO, 50% EtOH, and H_2O respectively, using the values of k_1+k_7 and t_m. From Eq. (6), the maximum value of $[\mathrm{M_E}]$ at t_m ($[\mathrm{M_E}]_\mathrm{m}$) is given by:

$$[\mathrm{M_E}]_\mathrm{m} = (k_1/k_6)[\mathrm{E}]_0\exp\{-(k_1+k_7)t_\mathrm{m}\}$$

So that the maximum value of Δ_{395}(Δ_m) is given by:

$$\Delta_\mathrm{m} = \varepsilon_{395}(k_1/k_6)[\mathrm{E}]_0\exp\{-(k_1+k_7)t_\mathrm{m}\} \quad (7)$$

From Eqs. (5) and (7), we can finally write:

$$\Delta_{395} = [k_6\Delta_\mathrm{m}/(k_1+k_7-k_6)\exp\{-(k_1+k_7)t_\mathrm{m}] \times [\exp(-k_6t) - \exp\{-(k_1+k_7)t\}] \quad (8)$$

From the curves shown in Fig. 2, Δ_m being found to be 45×10^{-2}, 11×10^{-2}, and 4×10^{-2} in 50% DMSO, 50% EtOH, and H_2O respectively, the Δ_{395} values in these media can be calculated by the following numerical equations:

In 50% DMSO;

$$
\begin{aligned}
\Delta_{395} &= \{0.10\times 45\times 10^{-2}/(0.14-0.10)\exp(-0.14\times 8)\} \\
&\qquad \times\{\exp(-0.10t)-\exp(-0.14t)\} \\
&= 3.45\{\exp(-0.10t)-\exp(-0.14t)\} \qquad (9)
\end{aligned}
$$

In 50% EtOH:

$$
\begin{aligned}
\Delta_{395} &= \{0.13\times 11\times 10^{-2}/(0.05-0.13)\exp(-0.05\times 12)\} \\
&\qquad \times\{\exp(-0.13t)-\exp(-0.05t)\} \\
&= -0.325\{\exp(-0.13t)-\exp(-0.05t)\} \qquad (10)
\end{aligned}
$$

In H_2O:

$$
\begin{aligned}
\Delta_{395} &= \{1.1\times 4\times 10^{-2}/(0.19-1.1)\exp(-0.19\times 2)\} \\
&\qquad \times\{\exp(-1.1t)-\exp(-0.19t)\} \\
&= -0.07\exp\{(-1.1t)-\exp(-0.19t)\} \qquad (11)
\end{aligned}
$$

In Table 1, the value of Δ_{395} in 50% DMSO, and 50% EtOH calculated using Eqs. (9) and (10) are tabulated, together with the observed values in the media. It can be seen from the table that the calculated Δ_{395} values are in fairly good agreement with the observed Δ_{395} values. The observed values of Δ_{395} in H_2O were, however, too small to make such a comparison.

Assuming that the value of ε_{395} does not appreciably vary in these media, we can calculate the ratio of k_1 (the generation rate of M_E) in the media from Eq. (7):

$$
\begin{aligned}
&k_1(\text{DMSO}) : k_1(\text{EtOH}) : k_1(H_2O) = 0.10\times 45\times 10^{-2}/ \\
&\quad \exp(-0.14\times 8) : 0.13\times 11\times 10^{-2}/\exp(-0.05\times 12) \\
&\quad : 1.1\times 4\times 10^{-2}/\exp(-0.19\times 2) = 5.3 : 1 : 2.47 \qquad (12)
\end{aligned}
$$

TABLE 1. OBSERVED AND CALCULATED VALUES OF Δ_{395} IN 50% DMSO AND 50% EtOH

Reaction time (min)	50% DMSO		50% EtOH	
	Δ_{395} (obsd)	Δ_{395} (calcd)	Δ_{395} (obsd)	Δ_{395} (calcd)
2	10×10^{-2}	22.4×10^{-2}		
4	31	34.1		
5			8×10^{-2}	9×10^{-2}
6	41	40.3		
8	45	42.4		
10	44	41.7	10.5	10.8
12	41	39.7	11	11.2
14	37	36.6		
16	34	33.1		
18	30	29.3		
20	26	25.5	9	9.7
22	24	22.4		
24	21	19.3		
26	18	16.5		
28	16	14.1		
30	14	12.0	6	6.7
40			3.5	4.1
50			2	2.6

On the other hand, as Eq. (2) indicates that:

$$I_{E_0} = \{k_1k_4/(k_4+k_4')\}[E]_0$$

at $t=0$, the relative values of I_{E_0} in 50% DMSO, 50% EtOH, and H_2O can be estimated to be 87, 17, and 14 respectively by the extrapolation of the log plot of I_E (shown as relative intensities in Fig. 4) to zero-time. The ratio of the apparent efficiencies of the FL-emission ($k_4/(k_4+k_4')$) in the media was found to be 75 : 73 : 25, so we can also calculate the ratio of k_1 as follows:

$$
\begin{aligned}
k_1(\text{DMSO}) : k_1(\text{EtOH}) : k_1(H_2O) &= 87/75 : 17/73 : 14/25 \\
&= 5.0 : 1 : 2.43 \qquad (13)
\end{aligned}
$$

The ratio in Eq. (13) is in good agreement with that in Eq. (12).

All of the results provide additional support for the view that the blue-fluorescent species is the primary excited product.

Unfortunately, the quantity of the isolated blue-fluorescent product was too small for it to be weighed or identified. 3,5-Dibromo-2,4-dihydroxybenzoate-2, which was produced when eosin Y was hydrolized in a 50% aqueous solution of sodium hydroxide for five hours at 140 °C,(6) showed no fluorescence at 480 nm. The fluorescence spectrum of 3,6-dihydroxyxanthone, which has been identified as the emitting species in the chemiluminescent reaction of succinylfluorescein, showed a peak at 410 nm.(7) The question as to the identification of the blue-fluorescent product, therefore, remains unanswered.

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2) The change of the spectrum in UV region (below 350 nm) could not be measured because the absorption of hydrogen peroxide retarded the measurement.

3) I. Kamiya and R. Iwaki, This Bulletin, **39**, 257, 264, 269, 277 (1966).

4) It was also found in the previous study,(1) that the FL-emission (a peak at 480 nm) which appeared during the course of the reaction decreased by successive oxidation.

5) The CL-emission intensity of xanthene dyes with hydrogen peroxide was about 100 times less than that of xanthene dyes with ozone whose quantum yield was reported to be $10^{-3}\sim 10^{-4}$ einstein/mol (J. Nikokavouras and G. Vassilopoulos, *Z. Phys. Chem. (N.F.)*, **85**, 205 (1973).).

6) G. Heller, *Chem. Ber.*, **28**, 312 (1895); R. Meyer and H. Meyer, *ibid.*, **29**, 2623 (1896).

7) I. Kamiya and K. Aoki, This Bulletin, **47**, 1744 (1974).