Mechanism of the Photosensitized Chemiluminescence of Luminol

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The photosensitized chemiluminescence (CL) of luminol in basic aqueous solution has been investigated by pulsed laser irradiation and by steady state light irradiation in order to clarify the CL in terms of Type-I (electron transfer) and Type-II (singlet oxygen) mechanisms. Short-lived (μs) and long-lived (ms) CL were observed at 420 nm upon pulsed laser irradiation near 530 nm of the luminol/sensitizer reaction mixture. The kinetics of the CL decay is explained by four rate constants,

$$I(t) = A_{ex}\{\exp(-k_2 t) - \exp(-k_1 t)\} + B_{ex}\{\exp(-k_4 t) - \exp(-k_3 t)\}.$$

These results, in addition to the results under steady state conditions, suggest that the long-lived CL dominates the photosensitized CL, which is caused by electron transfer to the triplet state of the sensitizers from luminol (Type-I). On the other hand, short-lived CL is due to a reaction between singlet oxygen and luminol (Type-II), the efficiency of which is smaller by two orders of magnitude compared to that of the Type-I mechanism.

The chemiluminescence (CL) of luminol is a well-known reaction used for the detection of bloodstain. The CL occurs from the singlet excited state of 3-aminophthalate ion (APA^*), formed by the oxidation of luminol.[1] Photosensitized CL of luminol was first demonstrated by Kuschnir and Kuwana.[2] They observed blue emissions upon the irradiation of a basic aqueous solution of luminol in the presence of fluorescein (Flu) or Eosin Y (Eo) as a photosensitizer. They have suggested that the CL is initiated by an energy transfer from an excited triplet state of photosensitizer ($^3M^*$) to molecular oxygen ($^3O_2$) to form singlet oxygen ($^1O_2^*$), which then reacts with luminol. The thus-produced endoperoxide decomposes to the active species APA^*.[3]

The energy-transfer process observed in the above reaction is commonly referred to as a Type-II mechanism.[4] There is another photooxidation mechanism, known as a Type-I, which is initiated by electron transfer from the substrate molecule, like luminol to $^3M^*$. Although the first photosensitized CL of luminol was explained by the Type-II mechanism, solid experimental evidence has not been reported so far to our knowledge. Most of the reactions are rather considered to proceed via the Type-I mechanism instead.

Matheson and Lee found that the reaction of luminol with $^1O_2^*$ generated directly upon pulsed-laser irradiation does not yield CL.[5] Their results denied the Type-II process, at least for the high concentrations of $^3O_2$ that they used to produce a sufficient amount of $^1O_2^*$ in the system. Klimov et al. have performed the CL of luminol or luminol derivatives by dye-sensitized photooxidation.[6] They used Methylene Blue (MB) and Eo as a photosensitizer, and showed that the photosensitized CL proceeded through a reaction of the luminol radical and superoxide (O_2^-). The luminol radical was generated by a Type-I electron transfer to the $^3M^*$ and the thus-generated dye-anion gave an electron to $^3O_2$ to form O_2^-. Recently, Motsenbocker et al. added a free-radical quencher (the quencher of the Type-I reaction) or $^1O_2^*$ quencher (the quencher of the Type-II reaction) to the dye-sensitized photooxidation of luminol.[7] Since the CL was only suppressed by the free-radical quencher, they concluded that the photosensitized CL was due to the Type-I reaction between luminol and $^3M^*$.

However, the above evidence seems not to be sufficient to completely deny the Type-II pathway in the photosensitized CL reaction. For example, Motsenbocker et al. ignored the possibilities that the radical quencher reacts with the other luminol intermediates which lead to CL, besides the luminol radical. We consider that the both of Type-I and Type-II reactions simultaneously occur in the photosensitized CL of luminol. To study the nature of the photosensitized CL, it is crucial to separate the Type-I and Type-II reactions in the system and to clarify the CL efficiency of each pathway. In the work, we studied, for the first time, the dynamics of the photosensitized CL of luminol by time-resolved CL measurements, and explained the CL reaction in terms of Type-I and Type-II mechanisms.

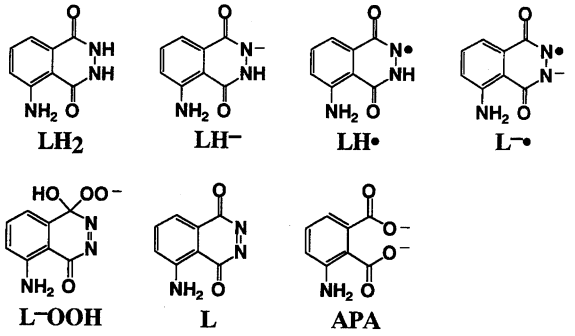
Experimental

Reagent-grade Eosin Y (Eo) and Rose Bengal (RB) from Wako Pure Chemical Ltd. and Luminol (97%) from Sigma Chemical Company were used without further purification. NaOH was 97% pure reagent grade from Kanto Chemical Co., Inc. Distilled water was treated with Mill-Q Labo (Nihon Millipore Ltd.) prior to use to eliminate any trace of metal ions. Mixed aqueous NaOH solutions of luminol and the photosensitizer near pH 13 were also freshly prepared. The pH values of sample solutions were measured with a Hanna pH meter (B417). All of the reactions were performed in air using quartz cuvettes (1 cm×1 cm×4 cm) at room temperature.

For chemiluminescence measurements in the μs and ms range, a dye laser (Lambda physick FL 2002, FWHM: 30 ns, 0.15 mJ/pulse) excited by a XeCl excimer laser (Lambda physick EMG 103) was used as a light source. The excitation wavelength was changed to match the optimum excitation wavelength of each photosensitiser near 530 nm. A bandpass filter at 400 nm (Toshiba V-V40) and a monochromator were used to cut any unnecessary light. The emission at 420 nm was collected on a photomultiplier (PM) (Hamamatsu R928). The PM signal was monitored by a digital oscilloscope (Tektronix TDS 520) and then transferred to a PC for storage, display and data analysis. Chemiluminescence spectra under photostationary conditions were measured with a Hitachi 850 fluorescence spectrophotometer with a cut-off filter at 480 nm (Toshiba V-Y48) and a band-pass filter at 440 nm (Toshiba V-V44). Absorption spectra were measured with a Hitachi 228A spectrophotometer. The abbreviations of the chemical species used in this paper are listed in Scheme 1.

Results

Figure 1 shows the rise-decay profile in the range of 0—1.4 ms of the CL with RB observed at 420 nm upon pulsed-laser irradiation near 530 nm with the initial molar concentration of luminol $[LH^-]_0 = 2.0\times10^{-4}$ M (1 M = 1 mol dm^{-3}) (luminol is present in the monoanion form (LH^-) at pH values used in these experiments[8]). The spectra of the CL are identical to the spectra of chemically-induced CL, for example, in the Hemin–H_2O_2–luminol system. It is obvious that the CL



Scheme 1.

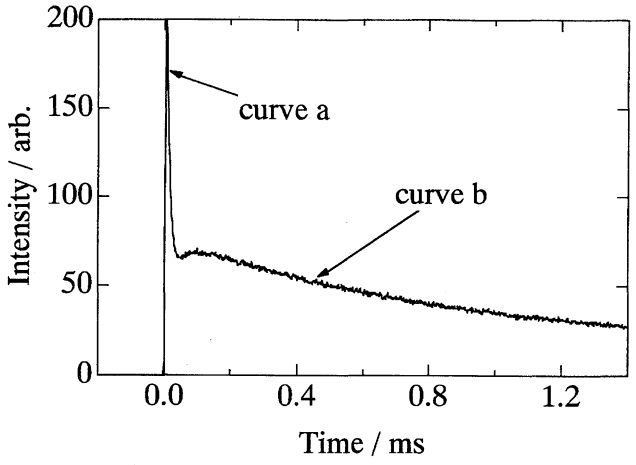


Fig. 1. The time profile in the range of 0—1.4 ms of photosensitized CL (monitored at 420 nm) using RB (1.8×10^{-5} M) upon a pulsed-laser irradiation at 530 nm at pH = 13. $[LH^-]_0 = 2.0\times10^{-4}$ M.

emission consists of a fast rise-decay component (curve a) and a relatively slow rise-decay one (curve b). The decay profile may be expressed as

$$I(t)=A_{ex}\{\exp(-k_2t)-\exp(-k_1t)\}+B_{ex}\{\exp(-k_4t)-\exp(-k_3t)\}, \quad (1)$$

where the first and the second terms represent the fast rise-decay and the slow rise-decay components, respectively.

Since the decay profile depends on $[LH^-]_0$ as well as the kind of photosensitizer, we measured the decay curves under various conditions. Figure 2 shows the rise-decay profile in the range of 0—28 μs of the photosensitized CL of luminol when Eo and RB were used as the sensitizers with $[LH^-]_0$ of (a) 5.5×10^{-3}, (b) 2.3×10^{-3}, (c) 8.2×10^{-4}, and (d) 1.0×10^{-4} M. The concentrations of Eo and RB were fixed at 5.7×10^{-6} M. Although the decay profiles varied according to both the sensitizers used and $[LH^-]_0$, in all cases, the CL emission rose rapidly within a few μs. Using these decay curves, we can obtain the rate constants as $k_1 = (2.8\times10^5+1.1\times10^8[LH^-]_0\ M^{-1})\ s^{-1}$, $k_2 = 1.7\times10^5\ s^{-1}$, $k_3 = 2.1\times10^4\ s^{-1}$, $k_4 = 4.0\times10^2\ s^{-1}$. The ratio of A_{ex} to B_{ex} depends on both $[LH^-]_0$ and the sensitizers used.

The rate constant, $k_1 = (2.8\times10^5+1.1\times10^8[LH^-]_0\ M^{-1})\ s^{-1}$, corresponds well to the decay rate constant of the singlet oxygen ($^1O_2^*$) observed from the $^1O_2^*$ emission at 1270 nm.[5,9—11] Assuming that the photosensitized CL proceed via Type-I (electron transfer) and Type-II (singlet oxygen) mechanisms, the first term in Eq. 1 (fast decay) may be considered to be due to the Type-II mechanism, and the second term in Eq. 1 (slow decay) should be due to the Type-I mechanism, accordingly.

Figure 3 shows the peak intensities, I_{pf}, of the CL emission in the fast decay curves (corresponding to curve a in Fig. 1) and the intensities, I_{ps}, in the slow decay curves at $t = 0.1$ ms (curve b) as a function of $[LH^-]_0$. The I_{pf} of the fast component increases initially and then decreases gradually along with an increase in $[LH^-]_0$. On the other hand, I_{ps} increases smoothly and approaches a constant value along with an increase in $[LH^-]_0$. A plot of $1/I_{ps}$ versus $1/[LH^-]_0$ (Stern–Volmer (S–V) type plot) is linear and the normalized slope (slope/intercept) shows quantitatively good agreement with the results of the S–V plot for the CL emissions at a steady state irradiation, which also shows good linearity (Fig. 4). In Table 1 summarizes the normalized slopes for the photosensitized CL using Eo and RB.

To examine the Type-I mechanism in more detail, we checked the reaction of luminol with the photosensitizer alone. Figure 5 shows the phosphorescence decay rates of RB as a function of $[LH^-]_0$. The triplet decay rate constant, k_p, showed a linear $[LH^-]_0$ dependence, $k_p = (4.2\times10^5 + k_{elt}[LH^-]_0)\ s^{-1}$ with $k_{elt} = 1.1\times10^9\ M^{-1}s^{-1}$ and $2.0\times10^9\ M^{-1}s^{-1}$ for RB and Eo, respectively, clearly indicating a direct reaction of the luminol and photosensitizer. The origin of the large k_{elt} value may be an electron transfer from the ground state of LH^- to the lowest excited triplet state of the sensitizer ($^3M^*$). The possibility of an energy transfer

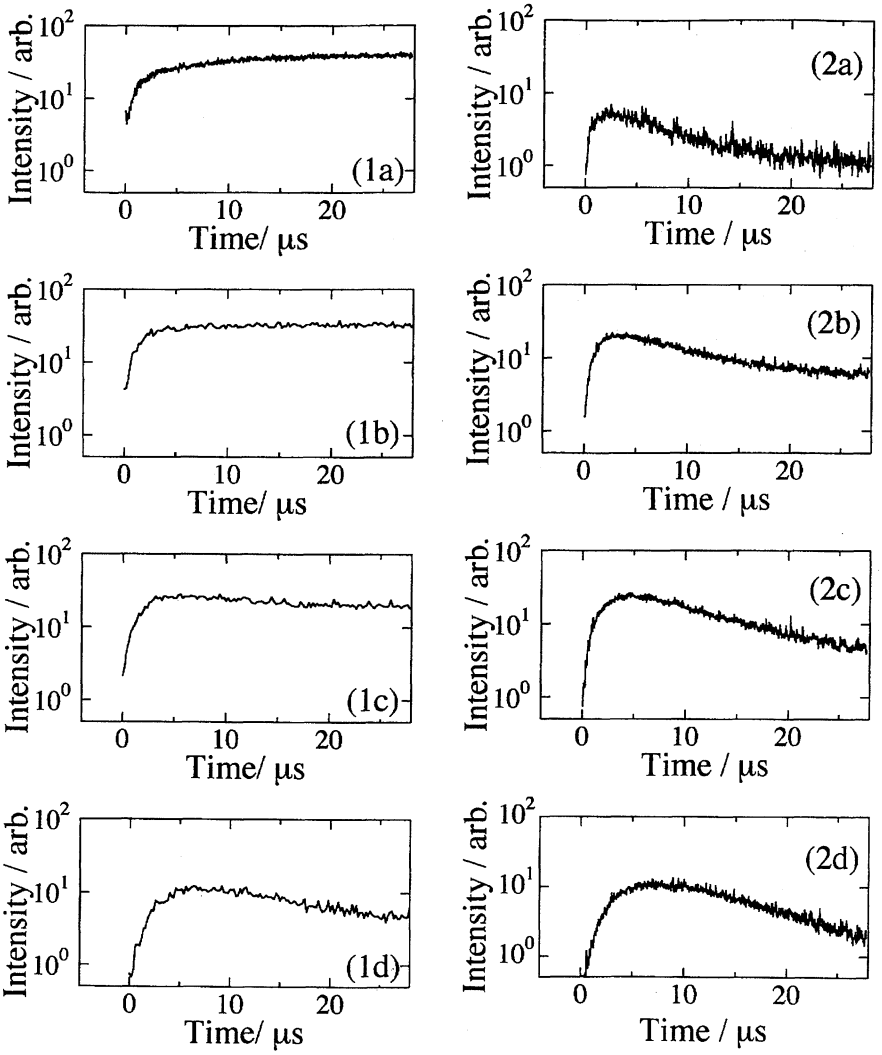


Fig. 2. The time profile in the range of 0—28 μs of photosensitized CL (monitored at 420 nm) upon the pulsed-laser irradiation at 530 nm. Sensitizers are (1) Eo (5.7×10^{-6} M) and (2) RB (5.7×10^{-6} M). The initial concentrations of luminol are (a) 5.5×10^{-3}, (b) 2.3×10^{-3}, (c) 8.2×10^{-4}, and (d) 1.0×10^{-4} M.

from the $^3M^*$ to triplet state of luminol ($^3LH^{-*}$) may be low, which is explained as follows. The S–T separations of a molecule having a relatively low-lying (π,π^*) state are generally around 5×10^3 cm^{-1}. Since the S_1 state of LH^- lies at around 2.3×10^4 cm^{-1}, the $^3LH^{-*}$ state appears to be higher than 1.8×10^4 cm^{-1} (shorter than 560 nm). The T_1 states of RB and Eo are located, respectively, near 1.5×10^4 cm^{-1} and 1.4×10^4 cm^{-1}. Consequently, a T–T energy transfer from $^3M^*$ to $^3LH^{-*}$ is impossible in the present system.

Therefore, the electron transfer from LH^- to $^3M^*$ is most probable for the origin of k_{elt}. This is consistent with the chemiluminescence mechanism of luminol without photo irradiation.[12—14] It is also reasonable from the viewpoints of the photosensitized oxidative-reductive reaction.[15—18] That is, the electron transfer from LH^- to $^3M^*$ is thermodynamically favorable, and the oxidation potential near 0.87 V (NHE) of LH^- corresponds to $k_{elt}\approx10^9$ $M^{-1}s^{-1}$.

Discussion

In the rise-decay profile in Fig. 1 there exist two kinds of components, fast and slow, which should correspond to Type-II and Type-I mechanism, respectively. However, before going into the kinetics, we first discuss the mechanism and intermediates of each reaction.

The Mechanism of Type-I Reaction. The reaction starts with the formation of an excited triplet state of the photosensitizer ($^3M^*$) through a photoexcited singlet state ($^1M^*$). The Type-I mechanism is initiated by an electron transfer from luminol (LH_2) to $^3M^*$. Under the present conditions, luminol is in the monoanion form (LH^-) and the anion radical ($L^{-\bullet}$) is formed by this reaction, since the LH· is thermodynamically unfavorable form ($pK_a = 7.7$).[19]

The $L^{-\bullet}$ can react either with O_2^- or $^3O_2$.[6,12—14] Merényi et al. and Klimov et al. have reported that the CL occurs through the reaction of $L^{-\bullet}$ and O_2^- to form L^-OOH as a key step.[20] The reaction with O_2^- is, however, unplausible in the present case. The formation of O_2^- in a photosensitized reaction is mainly due to an electron transfer from the semireduced dye (M^-) to $^3O_2$.[6,21] Although semireduced Eo and RB are known to react with $^3O_2$ rapidly ($k\approx10^8$ M^{-1} s^{-1}) to gen-

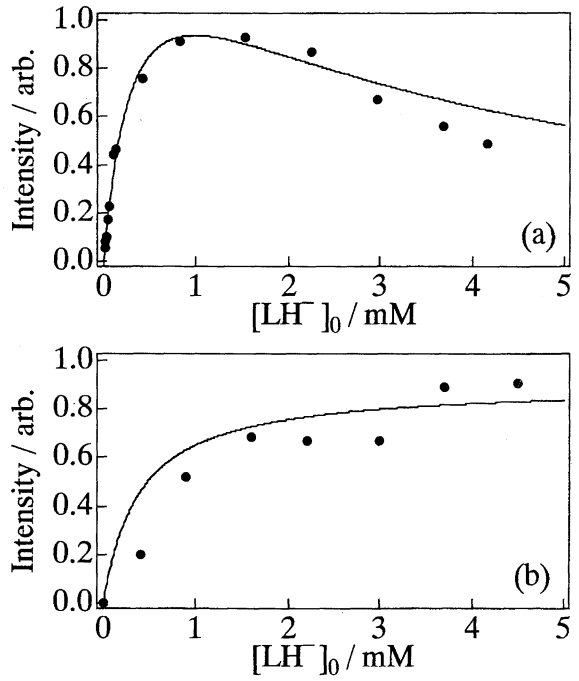


Fig. 3. (a) The peak intensities I_{pf} of the CL emission in fast decay curves (corresponding to curve a in Fig. 1) and (b) the intensities I_{ps} of the CL in slow decay curves at $t = 0.1$ ms (curve b) as a function of the luminol concentration at pH = 13. The sensitizer is RB (5.7×10^{-6} M). The theoretical curves calculated from Eqs. 8, 9, 10, and 11 are indicated.

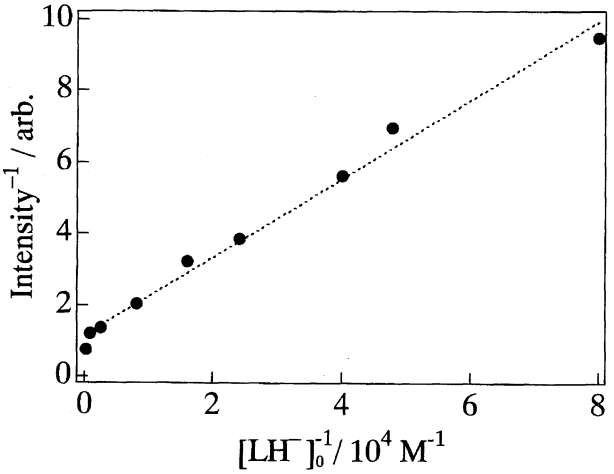


Fig. 4. Stern–Volmer plots of the intensity of photosensitized CL (monitored at 420 nm) upon the steady state irradiation (I_{ST}) at 530 nm at pH = 13. The sensitizer is RB (5.7×10^{-6} M).

erate O_2^-, the concentrations of $L^{-\bullet}$ and O_2^- after pulsed laser irradiation (0.15 mJ/pulse) are at most 10^{-10} M under the present experimental conditions. Since the $^3O_2$ concentration ($[^3O_2]_0$) under the present conditions is about 2×10^{-4} M, the reaction of $L^{-\bullet}$ with $^3O_2$ ($k_{r1}^0 = 9.8\times10^8$ M^{-1} s^{-1}) is dominant compared with the reaction of $L^{-\bullet}$ with O_2^- ($k = 2.3\times10^8$ M^{-1} s^{-1}). Therefore, we can safely conclude that this photosensitized CL due to a Type-I reaction proceeds via the $L^-OO\cdot$ formed by the reaction between $L^{-\bullet}$

Table 1. The Normalized Slope of Stern–Volmer Plot (ΔS–V, in M)

	$\Delta S-V_{ST}$ [a]	$\Delta S-V_{ps}$ [b]	$(k_p^0+k_d[^3O_2]_0)/k_{elt}$ [c]
RB	2.7×10^{-4}	2.5×10^{-4}	3.8×10^{-4}
Eo	1.6×10^{-4}	2.3×10^{-4}	2.1×10^{-4}

a) The normalized slope of S–V plot ($1/I_{ST}$ vs. $1/[LH^-]_0$) in the steady state experiments. b) The normalized slope of S–V plot ($1/I_{ps}$ vs. $1/[LH^-]_0$) of the slow CL in the pulsed-laser irradiation experiments. c) The values are obtained from the measurements of the triplet decay of photosensitizers.

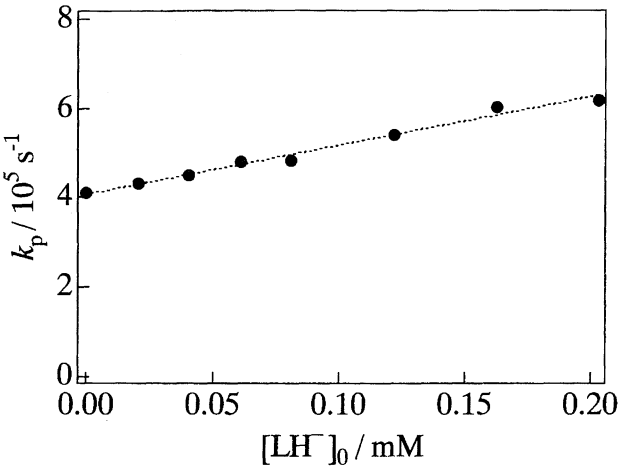


Fig. 5. The phosphorescence lifetime of RB (5.7×10^{-6} M, excited at 530 nm and monitored at 720 nm) at pH = 13 as a function of luminol concentration.

with $^3O_2$. The $L^-OO\cdot$ will be transformed to an intermediate, X.[13] The structure of X is not presently clear, although Baxendale suggested that X is produced by the elimination of nitrogen from $L^-OO\cdot$. The aminophtalate in the excited state (APA*), which finally emits light, is formed from X. A possible route to the APA*, so far proposed, is a bimolecular reaction of X with a second-order rate constant of $k = 3.9\times10^8$ M^{-1} s^{-1}. However, as mentioned above, the concentration of $L^{-\bullet}$, and thus that of X after pulsed laser irradiation, is less than 10^{-10} M. Under these conditions, the CL lifetime should be more than 0.1 s, unless other reaction paths of X exist. In order to remove this discrepancy, we introduced a unimolecular reaction path to explain the mechanism of photosensitized CL of Type-I:

$$L^{-\bullet} + {}^3O_2 \longrightarrow L^-OO\cdot \quad (2)$$

$$L^-OO\cdot \longrightarrow X \quad (3)$$

$$X \longrightarrow APA^* \quad (4)$$

$$APA^* \longrightarrow APA + h\nu \quad (5)$$

The Type-I reaction should be described by the slow component in Eq. 1 with $k_3 = 2.1\times10^4$ s^{-1} (rise) and $k_4 = 4.0\times10^2$ s^{-1} (decay). The reaction rate of Eq. 2 is around $k_{r1}^0[^3O_2]_0 \approx 2\times10^5$ s^{-1} under the present conditions. The decay rate constant of the $L^-OO\cdot$ is already reported by Hass and Würzberg as 2.5×10^4 s^{-1}.[22] Since the latter value is in

good agreement with k_3, we consider that the rise in the slow component of Eq. 1 corresponds to reaction (3), and that the slow decay should be due to the unimolecular reaction (4). Here, it is noted that the rate constant of reaction (5) is quite large.[22]

There are some arguments that the phthalazine-1,4-dione (L), which can be produced by the reaction of $L^{-\bullet}$ with $^3O_2$, also leads to CL.[12,23–26] However, the CL from the L pathway is negligible under our conditions, since the formation of L is a minor reaction (the rate constant of this reaction (550 $M^{-1}\,s^{-1}$) is much smaller than $k^0_{r1}[^3O_2]_0$).

The Mechanism of Type-II Reaction. The Type-II reaction is initiated by the energy transfer from $^3M^*$ to $^3O_2$ to form singlet oxygen ($^1O_2^*$), which reacts with luminol. The Type-II reaction should correspond to the fast term in Eq. 1 with $k_1 = (2.8\times10^5 + 1.1\times10^8[LH^-]_0\ M^{-1})\ s^{-1}$ and $k_2 = 1.7\times10^5\ s^{-1}$. As mentioned before, the rate constant k_1 is in good agreement with the decay rate of $^1O_2^*$. We can assume the reaction path of Type-II mechanism as follows:

$$LH^- + {}^1O_2^* \longrightarrow L^-OOH \tag{6}$$

$$L^-OOH \longrightarrow APA^* \tag{7}$$

The rise in the fast component in Eq. 1 corresponds to reaction (6). Although Kuschnir and Kuwana considered the intermediate as an endoperoxide,[2] we adopted here the L^-OOH instead, because the decay constant of the L^-OOH ($k_{r4} = 1.7\times10^5\ s^{-1}$) reported by Merényi et al. coincides with k_2.[20] The decay component should be due to reaction (7), accordingly. It should be noted that the L^-OOH is also produced by the reaction of $L^{-\bullet}$ and O_2^-. This reaction is, however, unplausible under the present conditions, as mentioned above.

Kinetics in the Chemiluminescence Due to Type-I and Type-II. The whole scheme of the photosensitized CL reaction is shown in Fig. 6 according to the discussion above. At least three intermediates are involved in a relatively slow Type-I process after the formation of $^3M^*$. On the other hand, in Type-II mechanism, one intermediate is involved after the formation of $^1O_2^*$.

The CL intensity due to Type I ($I(t)_{type\ I}$) and Type II ($I(t)_{type\ II}$) are expressed as follows:

$$I(t)_{type\ II} = A\{\exp(-k_{r4}t) - \exp(-k_O t)\}, \tag{8}$$

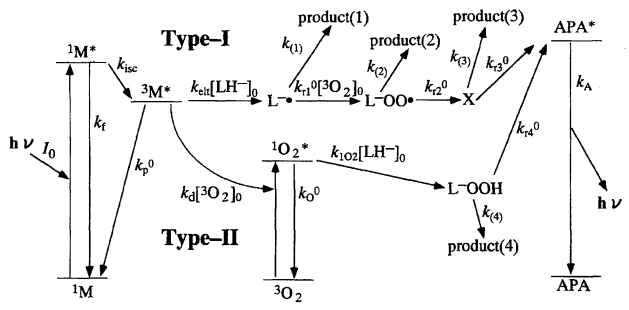


Fig. 6. Type-I and Type-II photosensitized CL reactions of luminol.

$$I(t)_{type\ I} = B\{\exp(-k_{r3}t) - \exp(-k_{r2}t)\}, \tag{9}$$

where $I(t) = I(t)_{type\ II} + I(t)_{type\ I}$. Using the scheme in Fig. 6, we can represent the coefficients A and B as follows (see Appendix):

$$A = \frac{c[LH^-]_0}{(a+(k_{elt}-k_{1O2})[LH^-]_0)(b+k_{1O2}[LH^-]_0)}, \tag{10}$$

$$B = \frac{e[LH^-]_0}{(d+k_{elt}[LH^-]_0)}. \tag{11}$$

With an increase in $[LH^-]_0$, A increases initially and then decreases gradually, while B increases approaching a constant value with high concentrations of $[LH^-]_0$. The theoretical results are shown in Fig. 3 by solid curves using the obtained k_{elt} and k_{1O2}. As can be seen in Fig. 3, the agreement between theory and experiment is satisfactory. We can thus conclude that the slow component in Eq. 1 corresponds to the CL emission due to the Type-I mechanism, and that the fast component corresponds to the Type-II mechanism. The Type-II mechanism dominates the fast response of photosensitized CL. However, from a comparison of the integrated intensity of the fast and slow decay described in Fig. 1, we can estimate that the CL emission due to the Type-II mechanism is less efficient by two orders of magnitude than that of Type-I. These phenomena have also been confirmed in the CL using Eo and Flu as photosensitizers.

The intensity, I_{ST}, of the CL upon steady state irradiation has almost the same $[LH^-]_0$ dependence of Eqs. 10 and 11 for the Type-II and Type-I mechanism, respectively (see Appendix), as

$$I_{ST\ II} = \alpha\cdot\frac{k_d[^3O_2]_0}{k_p^0+k_d[^3O_2]_0+k_{elt}[LH^-]_0}\cdot\frac{k_{1O2}[LH^-]_0}{k_O^0+k_{1O2}[LH^-]_0}, \tag{12}$$

$$I_{ST\ I} = \beta\cdot\frac{k_{elt}[LH^-]_0}{k_p^0+k_d[^3O_2]_0+k_{elt}[LH^-]_0}. \tag{13}$$

The linear correlation between I_{ST}^{-1} and $[LH^-]_0^{-1}$ in Stern–Volmer (S–V) plots shown in Fig. 4 suggest that the CL emission upon steady state irradiation is attributed mostly to the Type-I mechanism. According to Eq. 13, the normalized slope of S–V plots is represented as $(k_p^0+k_d[^3O_2]_0)/k_{elt}$. The value of the normalized slope (2.7×10^{-4}) is in good agreement with the $(k_p^0+k_d[^3O_2]_0)/k_{elt}$ (3.8×10^{-4}), which is obtained separately by measurements of the triplet decay of the photosensitizers. The results are summarized in Table 1 together with those obtained using Eo as a photosensitizer. The Type-I mechanism is predominant in both the steady-state and transient CL with the photosensitizer of RB or Eo.

In conclusion, an electron transfer from luminol to the lowest excited triplet state of the sensitizer $^3M^*$ contributes mostly to the photosensitized CL (Type-I mechanism). However, a CL reaction between $^1O_2^*$ (Type-II mechanism) simultaneously occurs, which shows very fast decay. We consider that the mechanism discuss here is applicable to other CL systems, such as *Cypridina* luciferin analogues (CLAs), which is known as a detector for active oxygen species, such as O_2^-.[9,27,28] Studies on this line are now in progress.

Appendix

According to Fig. 6, the differential equations for each process of Type-II reaction are expressed as follows:

$$\frac{d[^3M^*]}{dt} = -k_p[^3M^*],$$
$$\frac{d[^1O_2]}{dt} = k_d[^3O_2]_0[^3M^*] - k_O[^1O_2],$$
$$\frac{d[L^-OOH]}{dt} = k_{1O2}[^1O_2][LH^-]_0 - k_{r4}[L^-OOH],$$
$$\frac{d[APA^*]}{dt} = k^0_{r4}[L^-OOH] - k_A[APA^*],$$

where

$$k_p = k^0_p + k_d[^3O_2]_0 + k_{elt}[LH^-]_0,$$
$$k_O = k^0_O + k_{1O2}[LH^-]_0,$$
$$k_{r4} = k^0_{r4} + k_{(4)},$$
$$k_A = k_{fA} + k_{nA}.$$

k_{fA} and k_{nA} are the radiative and non-radiative decay rate constants of APA*, respectively.

Using the conditions $k_A \gg k_p > k_O$, $k_f + k_{isc} \gg k_p$, and $k_p \gg k_{r4}$,[22] the concentration of APA* in the transient state ([APA*]) is simply represented as

$$[APA^*] = \frac{k_d k_{1O2} k^0_{r4}[LH^-]_0[^3O_2]_0[^3M^*]_0}{(k_p - k_O)(k_O - k_{r4})k_A}\{\exp(-k_{r4}t) - \exp(-k_O t)\}.$$

Here, we use the relation

$$[^3M^*]_0 = I_0 \frac{k_{isc}}{(k_f + k_{isc})},$$

where I_0 is the amount of absorbed light per second and ($k_{isc}/(k_f + k_{isc})$) represents the quantum yield of the intersystem crossing of ^{1}M* to ^{3}M*.

Since the time dependence of the CL intensity for the Type-II reaction ($I(t)_{type\ II}$) is the product of k_{fA} and [APA*], we can express the $I(t)_{type\ II}$ as

$$I(t)_{type\ II} = A\{\exp(-k_{r4}t) - \exp(-k_O t)\}, \quad (8)$$

where

$$A = \frac{c[LH^-]_0}{(a + (k_{elt} - k_{1O2})[LH^-]_0)(b + k_{1O2}[LH^-]_0)}. \quad (10)$$

Here,

$$a = k^0_p + k_d[^3O_2] - k^0_O,$$
$$b = k^0_O - k_{r4},$$
$$c = \frac{k_d k_{1O2} k^0_{r4} k_{fA}[^3O_2]_0[^3M^*]_0}{k_A}.$$

On the other hand, the differential equations for each process of the Type-I reaction are expressed as follows:

$$\frac{d[L^{-\bullet}]}{dt} = k_{elt}[LH^-]_0[^3M^*] - k_{r1}[L^{-\bullet}],$$
$$\frac{d[L^-OO\cdot]}{dt} = k^0_{r1}[^3O_2]_0[L^{-\bullet}] - k_{r2}[L^-OO\cdot],$$
$$\frac{d[X]}{dt} = k^0_{r2}[L^-OO\cdot] - k_{r3}[X],$$
$$\frac{d[APA^*]}{dt} = k^0_{r3}[X] - k_A[APA^*],$$

where

$$k_{r1} = k^0_{r1}[^3O_2]_0 + k_{(1)},$$
$$k_{r2} = k^0_{r2} + k_{(2)},$$
$$k_{r3} = k^0_{r3} + k_{(3)}.$$

Using the conditions $k_A \gg k_p$, $k_f + k_{isc} \gg k_p$, and $k_p > k_{r1} \gg k_{r2} \gg k_{r3}$, [APA*] for the Type-I reaction is simply represent as

$$[APA^*] = \frac{k_{elt}k^0_{r1}k^0_{r2}k^0_{r3}[LH^-]_0[^3O_2]_0[^3M^*]_0}{(k_p - k_{r1})k_{r1}k_{r2}k_A} \times\{\exp(-k_{r3}t) - \exp(-k_{r2}t)\}.$$

Then, the CL intensity for the Type-I reaction ($I(t)_{type\ I}$) is expressed as

$$I(t)_{type\ I} = B\{\exp(-k_{r3}t) - \exp(-k_{r2}t)\}, \quad (9)$$

where

$$B = \frac{e[LH^-]_0}{(d + k_{elt}[LH^-]_0)}. \quad (11)$$

Here,

$$d = k^0_p + k_d[^3O_2]_0 - k^0_{r1}[^3O_2] - k_{(1)},$$
$$e = \frac{k_{elt}k^0_{r1}k^0_{r2}k^0_{r3}k_{fA}[^3O_2]_0[^3M^*]_0}{k_{r1}k_{r2}k_A}.$$

The intensity of the CL of Type-II reaction under steady state irradiation ($I_{ST\ II}$) is represented as

$$I_{ST\ II} = [^3M^*]_0 \cdot \frac{k_d[^3O_2]_0}{k^0_p + k_d[^3O_2]_0 + k_{elt}[LH^-]_0} \times \frac{k_{1O2}[LH^-]_0}{k^0_O + k_{1O2}[LH^-]_0} \cdot \frac{k^0_{r4}}{k^0_{r4} + k_{(4)}} \cdot \frac{k_{fA}}{k_{fA} + k_{nA}}.$$

The $[LH^-]_0$ dependence of $I_{ST\ II}$ is

$$I_{ST\ II} = \alpha \cdot \frac{k_d[^3O_2]_0}{k^0_p + k_d[^3O_2]_0 + k_{elt}[LH^-]_0} \cdot \frac{k_{1O2}[LH^-]_0}{k^0_O + k_{1O2}[LH^-]_0}, \quad (12)$$

where

$$\alpha = [^3M^*]_0 \cdot \frac{k^0_{r4}}{k^0_{r4} + k_{(4)}} \cdot \frac{k_{fA}}{k_{fA} + k_{nA}}.$$

In the same way, the CL intensity of the Type-I reaction under steady state irradiation ($I_{ST\ I}$) is

$$I_{ST\ I} = \beta \cdot \frac{k_{elt}[LH^-]_0}{k^0_p + k_d[^3O_2]_0 + k_{elt}[LH^-]_0}, \quad (13)$$

where

$$\beta = [^3M^*]_0 \cdot \frac{k^0_{r1}[^3O_2]_0}{k^0_{r1}[^3O_2]_0 + k_{(1)}} \cdot \frac{k^0_{r2}}{k^0_{r2} + k_{(2)}} \cdot \frac{k^0_{r3}}{k^0_{r3} + k_{(3)}} \cdot \frac{k_{fA}}{k_{fA} + k_{nA}}.$$

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