# Mechanism of Photosensitized Chemiluminescence of 2-Methyl-6-phenylimidazo[1,2-a]pyrazin-3(7*H*)-one (CLA) in Aqueous Solution

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The photosensitized chemiluminescence (CL) of 2–methyl–6–phenylimidazo[1,2–*a*]pyrazin–3(7*H*)–one (CLA) in aqueous solution of Rose Bengal near pH 7 has been investigated. Three time profiles (fast decay, fast rise–and–decay, and very slow decay components) of CL were observed at 380 nm. The former two terms were due to the Type II and Type I reactions, respectively. The intermediates of the each reactions were apparently different.

Both singlet oxygen ($^1O_2$*) and superoxide ($O_2^-$) are important in various biological and chemical processes. For detecting those active oxygen species, the chemiluminescence (CL) of luminol or *Cypridina* Luciferin Analogues (CLAs) has been expected as one of the versatile tools.[1–6] Although there are many reports on the CL of CLAs, the properties and intermediates due to the reactions with $O_2^-$ or $^1O_2$* in water are not known well.[2–9] In this letter, we report, for the first time, the dynamics of the CL of a typical CLAs, 2–methyl–6–phenylimidazo[1,2–*a*]pyrazin–3(7*H*)–one (CLA), through the reaction with $O_2^-$ or $^1O_2$* induced by a photosensitized reaction. We used Rose Bengal (RB) as a photosensitizer since the excited triplet state of RB ($^3$RB*) is well–known as a generator of $^1O_2$* as well as an initiator of radical reaction.[10,11]

Reagent grade of RB from Wako Pure Chemical Ltd. and CLA (97%) from Tokyo Kasei Kogyo Co., Ltd. were used without further purification. Distilled water was treated with Mill–Q Labo (NIHON MILLIPORE Ltd.) prior to use to eliminate trace of metal ions. The aqueous phosphate buffer solution (pH 6.77) containing CLA ($[CLA]_0=1.9\times10^{-5}$ M, 1 M=1 mol dm$^{-3}$) and RB ($4.2\times10^{-5}$ M) was also prepared as fresh. The solution was bubbled with $N_2$ to regulate the concentration of $^3O_2$ and the initial concentration $[^3O_2]_0$ was determined from the lifetime of $^3$RB*. The CL decay was measured by the gated photoncounting detection at room temperature. A xenon flash lamp (>510 nm) with a pulse duration of 3 μs was used for the excitation. The emitted signals at 380 nm were collected on a photomultiplier (Hamamatsu R446).

Figure 1 shows the decay curves of the photosensitized CL of CLA in the range of 0 – 0.6 s with the $[^3O_2]_0$ of $2.0\times10^{-4}$, $1.0\times10^{-5}$ and $1.1\times10^{-6}$ M for curves (a), (b) and (c), respectively. The emission spectrum of the CL was identical to that of xanthine – xanthine oxidase – CLA system. Several features are seen in the decay curves. 1) A slow decay component with the lifetime of 0.5 s exists in each curves. 2) A relatively fast rise–and–decay component becomes prominent when the $[^3O_2]_0$ is low (curves (b) and (c)). 3) A fast decay component is predominant under high $[^3O_2]_0$ condition (curve (a))

These results suggest that the CL emission time profile consists of three independent processes, that is, fast decay component (fd), fast rise–and–decay component (rd), and very slow decay component (sd) as,

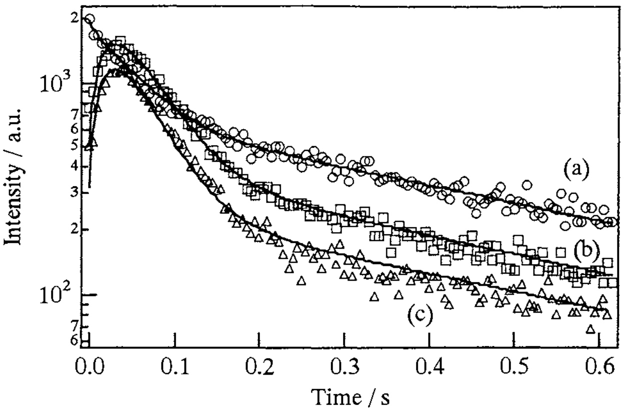

**Figure 1.** The time profile of the photosensitized CL of CLA with various concentration of $[^3O_2]_0$ ((a) $2.0\times10^{-4}$ M, (b) $1.0\times10^{-5}$ M, (c) $1.1\times10^{-6}$ M). $[CLA]_0=1.9\times10^{-5}$ M, $[RB]_0=4.2\times10^{-6}$ M. The solid curves are theoretical curves obtained from Eq. (1).

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

where $A_{fd}$, $B_{rd}$ and $C_{sd}$ are the constants. The deconvolution of the CL emission curve leads to the rate constants $k_1$ to $k_4$ as 30, 34, 28 and 2.0 s$^{-1}$, respectively. The fitting curves thus obtained are indicated by solid curves in Figure 1. The $[^3O_2]_0$ dependence of $A_{fd}$, $B_{rd}$ and $C_{sd}$ are shown in Figure 2.

The mechanism of the reaction may be represented as Figure 3.[5–7,12,13] The observed CL should be due to Type I (electron transfer) and Type II (singlet oxygen) process. In Figure 3, CLA$^+$,

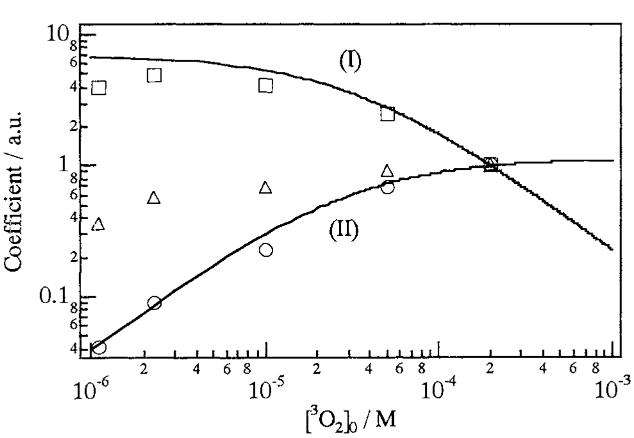

**Figure 2.** Plots of the $A_{fd}$ (○), $B_{rd}$ (□) and $C_{sd}$ (△) against $[^3O_2]_0$. Curves (I) and (II) are the theoretical curves for Type I (obtained from Eq. (3)) and Type II (obtained from Eq. (2)) reactions, respectively.

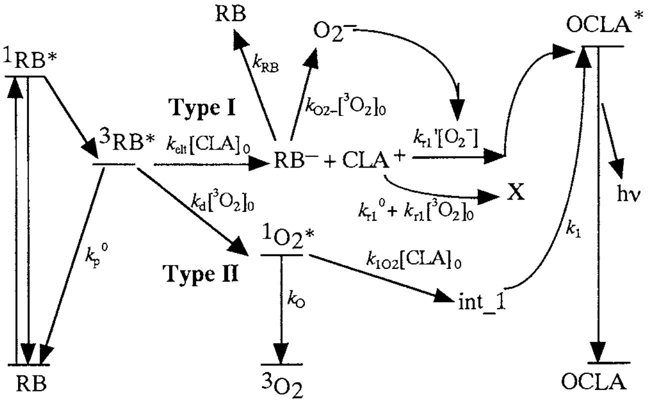

**Figure 3.** Type I and Type II photosensitized CL reactions of CLA.

$RB^-$, X and int_1 are radical cation of CLA, radical anion of RB, non–CL product and intermediate of CLA, respectively. The OCLA* is 3–acetamido–6–phenylpyrazine in the excited state which is the actual CL emitter. Based on Figure 3, the $[^3O_2]_0$ dependence of the CL intensity due to the Type II reaction ($I_{\mathrm{TypeII}}$) is expressed as,

$$I_{\mathrm{TypeII}} \propto \frac{k_d[^3O_2]_0}{k_p{}^0 + k_{\mathrm{elt}}[\mathrm{CLA}]_0 + k_d[^3O_2]_0}. \tag{2}$$

The rate constants $k_p{}^0$, and $k_d$ are already reported to be $6.7\times10^3\ \mathrm{s}^{-1}$ and $1.6\times10^9\ \mathrm{M}^{-1}\mathrm{s}^{-1}$, respectively.[10] The $k_{\mathrm{elt}}$ is obtained from the lifetime of $^3RB^*$ as $2.0\times10^9\ \mathrm{M}^{-1}\mathrm{s}^{-1}$. The theoretical curve from Eq. (2) is plotted in Figure 2 (curve (II)), which corresponds well with the $[^3O_2]_0$ dependence of $A_{\mathrm{fd}}$. Thus, we can conclude that the first term of Eq. (1) is due to Type II reaction. We consider in Figure 3 that at least one intermediate (int_1) must be involved after the generation of $^1O_2^*$ state in Type II reaction. The decay rate constants of OCLA*, $^3RB^*$ and $^1O_2^*$ are considerably large compared with $k_1$. Therefore, the int_1 should determine the decay rate of Type II reaction although we have not identified the int_1 yet.

On the other hand, we can not predict strictly the $[^3O_2]_0$ dependence of the CL intensity due to the Type I reaction ($I_{\mathrm{TypeI}}$) since the values of $k_{r1}{}^0$, $k_{r1}$ and $k_{r1}'$ in Figure 3 have not been obtained yet. However, $I_{\mathrm{TypeI}}$ is proportional to the amount of $CLA^+$ for Type I reaction. The yield of $CLA^+$ ($\phi_{\mathrm{CLA+}}$) is represented as,

$$\phi_{\mathrm{CLA+}} = \frac{k_{\mathrm{elt}}[\mathrm{CLA}]_0}{k_p{}^0 + k_{\mathrm{elt}}[\mathrm{CLA}]_0 + k_d[^3O_2]_0}. \tag{3}$$

According to Eq. (3), $\phi_{\mathrm{CLA+}}$ decreases with an increase in $[^3O_2]_0$. As shown in Figure 3, $B_{\mathrm{rd}}$ decreases with an increase in $[^3O_2]_0$, and the $[^3O_2]_0$ dependence of $B_{\mathrm{rd}}$ is very similar to that of $\phi_{\mathrm{CLA+}}$. Therefore, we can conclude that the second term of Eq. (1) is due to Type I reaction.

We have not identified yet the nature of the CL due to the third term of Eq. (1). However, the decay rate constant, $k_4$, is in good agreement with that of $O_2^-$ reacting with CLA, indicating that the CL is due to the reaction between CLA and $O_2^-$.[5,14]

In conclusion, the photosensitized CL of CLA is composed of three components, fast decay, fast rise and decay, and very slow decay. The former two components are due to Type II and Type I reaction, respectively, and the intermediates of Type I and Type II are apparently different to each other.

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