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# Substituent effects on the kinetics for the chemiluminescence reaction of 6-arylimidazo[1,2-*a*]pyrazin-3(7*H*)-ones (*Cypridina* luciferin analogues): support for the single electron transfer (SET)–oxygenation mechanism with triplet molecular oxygen

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**Abstract**—Kinetics of chemiluminescence reactions of 2-methyl-6-phenylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (**1c**, *Cypridina* luciferin analogue) and substituent effects of the 6-aryl group of derivatives **1** strongly suggest that the rate-determining step is a single electron transfer from an anion derived from **1** to a triplet molecular oxygen ($O_2$) in the oxygenation process.

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Marine bioluminescent organisms, such as the crustacean *Cypridina* (*Vargula*)[1,2] and the jellyfish *Aequorea*,[3] have their own imidazo[1,2-*a*]pyrazin-3(7*H*)-one (imidazopyrazinone) derivatives as a bioluminescent substrate. *Cypridina* luciferin, for instance, is an imidazopyrazinone having three appendages, (*S*)-2-butyl, 3-indolyl, and 3-(1-guanidino)propyl groups, at C2, C6, and C8, respectively. An imidazopyrazinone substrate reacts with triplet molecular oxygen ($O_2$) to give an electronically excited amidopyrazine derivative under regulation by a luciferin or apophotoprotein, resulting in light emission with a high efficiency.

For several decades, we and other groups have investigated chemiluminescence reactions of imidazopyrazinones with $O_2$ to establish the bioluminescence mechanism.[4–7] A plausible mechanism for the chemiluminescence reaction of 6-aryl-2-methylimidazopyrazinone (**1**), a *Cypridina* luciferin analogue, can be proposed (Scheme 1). Particularly, oxygenation of **1** to give a peroxide intermediate ($\mathbf{3}^-$) is suggested by Goto[1,5] in the chemiluminescence reaction of 2-methylimidazo-[1,2-*a*]pyrazin-3(7*H*)-one in diglyme, containing *t*-BuOK as a base. The process from **1** to $\mathbf{3}^-$ is triggered by deprotonation of **1** with a base. The successive single electron transfer (SET) from the resulting $\mathbf{2}^-$ to $O_2$ probably gives a radical $\mathbf{2}^\bullet$ and the superoxide anion $O_2^{\bullet-}$. Fast radical coupling of $\mathbf{2}^\bullet$ and $O_2^{\bullet-}$ followed by a rearrangement affords a dioxetanone intermediate ($\mathbf{4}^-$) via $\mathbf{3}^-$. Finally, thermal decomposition of $\mathbf{4}^-$ with the loss of carbon dioxide generates a singlet-excited state of amidopyrazine anion ($\mathbf{5}^{-*}$), which emits light, yielding 2-acetamide-5-arylpyrazine (**6**) via protonation of $\mathbf{5}^-$. The SET from $\mathbf{2}^-$ to $O_2$ is the most controversial step and is suggested to be rate determining.[1,5] However, there has been no systematic study to prove that the SET process exists.

To verify the reaction mechanism, particularly the involvement of SET, we investigated the kinetics of the chemiluminescence reactions of 2-methyl-6-phenylimidazopyrazinone (**1c**), its *para*-substituted phenyl derivatives [**1a**, Ar = $p$-$(CH_3)_2NC_6H_4$; **1b**, Ar = $p$-$CH_3OC_6H_4$; **1d**, Ar = $p$-$ClC_6H_4$; **1e**, Ar = $p$-$NCC_6H_4$; Scheme 1], and the 3-indoyl derivative (**1f**), a prototype model of *Cypridina* luciferin.[8] We report here that the oxygenation reaction of $\mathbf{2}^-$ is controlled by SET from $\mathbf{2}^-$ to $O_2$,

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*Keywords*: Chemiluminescence; Imidazopyrazinone; Oxygenation; Substituent effects; Single electron transfer; Reaction rate.

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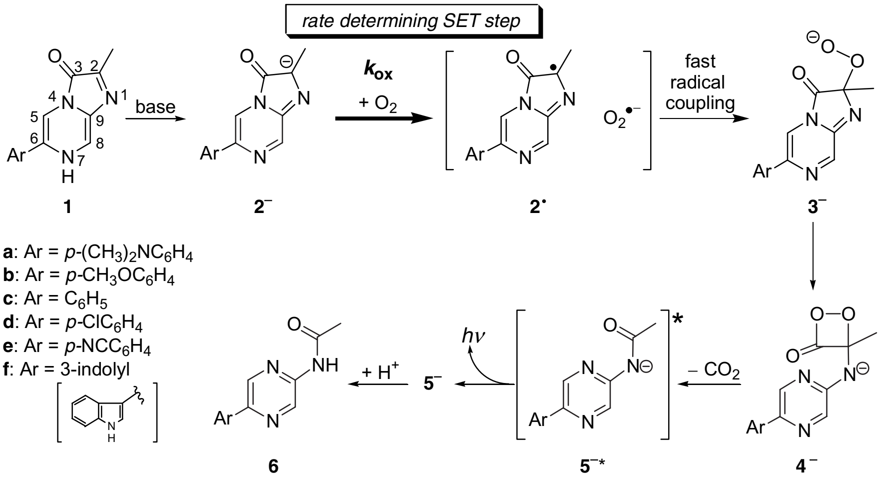

**Scheme 1.**

and the SET process is the rate-determining step in the whole chemiluminescence reaction of **1**.

First, we examined if 1,1,3,3-tetramethylguanidine (TMG) is an appropriate base for the kinetic study of **1** in acetonitrile. We considered TMG over *t*-BuOK because TMG, with a moderate basicity[9] and a good solubility in various organic solvents, is easier to handle. A small portion (20 μL) of a stock solution of **1c** ($1.0 \times 10^{-3}$ mol $L^{-1}$) in methanol was mixed with aerated acetonitrile[10] (2.0 mL) containing TMG at 25 ± 1 °C. The reactions of **1c** were traced by monitoring intensity (*I*) of the total emitted light,[11] which reached a maximum immediately after initiation of the reaction and then decayed monotonically (Fig. 1). The rate constant ($k_{obsd}$) of the pseudo-first-order decay was determined to be ca. $2.9 \times 10^{-2}$ $s^{-1}$.[12] Interestingly, no significant dependency on the concentration of TMG ([TMG] = 1–100 × $10^{-4}$ mol $L^{-1}$) was observed for (i) emission wavelengths from **5c**$^{-*}$ ($\lambda_{em} = 467$ nm), (ii) $k_{obsd}$, (iii) chemiluminescence quantum yields ($\Phi_{CL} = 1.0$–$1.1 \times 10^{-4}$), or (iv) chemical yields of the product, **6c** (78–91%).[13] Therefore, TMG acts as a suitable base to generate **2c**$^-$. It was also found that the reaction of **2c**$^-$ with $O_2$ proceeds at a constant rate.

Next, we examined the chemiluminescence reactions of **1c** in the presence of $O_2$ at various concentrations.[10] The reactions of **1c** were similarly traced in acetonitrile containing TMG (0.10 mol $L^{-1}$) at 25 ± 1 °C.[15] A linear correlation between $k_{obsd}$[12] and [$O_2$], $k_{obsd} = k_{ox}[O_2] + 0.00$, gave the second-order rate constant at 25 °C as a slope ($k_{ox} = 22$ $mol^{-1}$ L $s^{-1}$, Fig. 2).[16] These findings indicate that the rate constant is determined by the oxygenation reaction of **2c**$^-$ and $O_2$. This conclusion matches that of Goto.[5]

The $k_{ox}$ values for other substrates **1** are summarized in Table 1 together with $\lambda_{em}$ and $\Phi_{CL}$.[11–13,17] The $\log[k_{ox}(\mathbf{1})/k_{ox}(\mathbf{1c})]$ values are correlated to the Hammett $\sigma_p$ constant of the *p*-substituent[18] for **1a–e** (Fig. 3): the reaction was accelerated by an electron-donating group, predicting a possible operation of SET from **2**$^-$ to $O_2$ in the oxygenation process. To confirm this SET mechanism, we examined the correlation of the $k_{ox}$ values with the anodic peak potentials ($E^p_{ox}$) of **2**$^-$ measured with cyclic voltammetry in acetonitrile containing TMG (0.10 mol $L^{-1}$).[19] As shown in Table 1, the $k_{ox}$ of **1** continuously increased with lowering $E^p_{ox}$ values of **2**$^-$. This tendency matches the result of the Hammett correlation of the $\log[k_{ox}(\mathbf{1})/k_{ox}(\mathbf{1c})]$ versus $\sigma_p$. Judging from

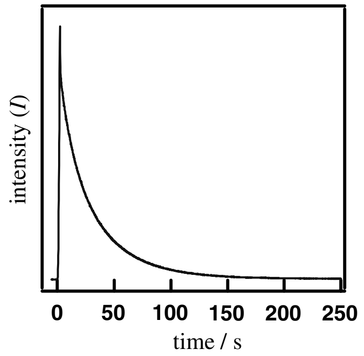

**Figure 1.** Intensity (*I*) of the total emitted light over time observed in the chemiluminescence of **1c** ($1.0 \times 10^{-5}$ mol $L^{-1}$) in aerated acetonitrile containing TMG (0.10 mol $L^{-1}$) at 25 ± 1 °C.

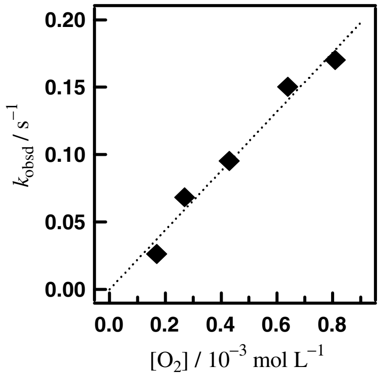

**Figure 2.** Correlation between the pseudo-first-order rate constants ($k_{obsd}$) for **1c** and concentrations of $O_2$ ([$O_2$]).

**Table 1.** Hammett constants ($\sigma_p$) and chemiluminescence data ($k_{ox}$, $\Phi_{CL}$, and $\lambda_{em}$) of **1** in acetonitrile containing TMG under air, oxidation potentials ($E_{ox}^{p}$) of anions $\mathbf{2}^-$ in acetonitrile, and the calculated maximum rate constants ($k_{et}$) of the SET from $\mathbf{2}^-$ to $O_2$

| Substrate [Ar] | $\sigma_p$ | $k_{ox}$[a] ($mol^{-1}\ L\ s^{-1}$) | $\Phi_{CL}$[b] $\times 10^{-4}$ | $\lambda_{em}$[c] (nm) | $E_{ox}^{p}$ *V* versus SCE | $k_{et}$ ($mol^{-1}\ L\ s^{-1}$) |
|---|---|---|---|---|---|---|
| **1a** [*p*-$(CH_3)_2NC_6H_4$] | −0.83 | 28 | 3.8 | 472 | −0.23 | 4.8 |
| **1b** [*p*-$CH_3OC_6H_4$] | −0.27 | 20 | 2.0 | 472 | −0.21 | 2.2 |
| **1c** [$C_6H_5$] | +0.00 | 17 | 1.0 | 467 | −0.19 | 1.0 |
| **1d** [*p*-$ClC_6H_4$] | +0.23 | 15 | 1.1 | 467 | −0.16 | 0.32 |
| **1e** [*p*-$NCC_6H_4$] | +0.66 | 11 | 0.8 | 468 | −0.13 | 0.10 |
| **1f** [3-indolyl] | — | 28 | 1.2 | 476 | −0.23 | 4.8 |

[a] The $k_{ox}$ values are observed oxygenation rate constants estimated to be $k_{obsd}/[O_2]$, where $[O_2] = 1.7 \times 10^{-3}\ mol\ L^{-1}$. The $k_{obsd}$ values were determined with the method described in Note 16.

[b] Chemiluminescence quantum yields.

[c] Emission maxima of the chemiluminescence spectra.

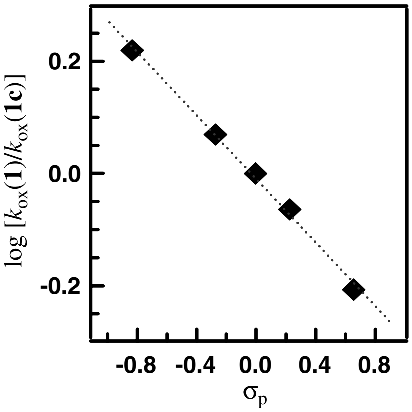

**Figure 3.** Hammett plot of the log[$k_{ox}$(**1**)/$k_{ox}$(**1c**)] values for **1a**–**e** against the $\sigma_p$ constant.

the estimated oxidation potentials ($E_{ox}^{1/2} = E_{ox}^{p} - 0.03$)[21] of $\mathbf{2}^-$ and the reduction potential of $O_2$ in acetonitrile ($E_{red}^{1/2} = -0.87$ V vs SCE),[20] the SET step is endergonic; the free energy change of SET from $\mathbf{2}^-$ to $O_2$ was calculated to be positive [$\Delta G_{et}^{0} = E_{ox}^{1/2} - E_{red}^{1/2} = +0.61 \sim +0.71\ eV > 0$]. When it was assumed that the activation free energy for SET is nearly equal to $\Delta G_{et}^{0}$, the maximum rate constant ($k_{et}$) of the SET step for each **1** was estimated to be 0.1–4.8 $mol^{-1}\ L\ s^{-1}$ (Table 1) by a theoretical treatment.[22–24] Therefore, the SET proceeds far more slowly than the radical coupling of $\mathbf{2}^{\bullet}$ and $O_2^{\bullet -}$, whose rate constant is expected to be over $10^8\ mol^{-1}\ L\ s^{-1}$, as is usual for alkyl radicals and $O_2^{\bullet -}$.[25]

While the $k_{et}$ values for **1** are considerably smaller than the corresponding $k_{ox}$ values, the $k_{et}$ values are linearly correlated to the $k_{ox}$ values, except for **1e** with the electron-deficient cyano group ($k_{et} = 0.34k_{ox} - 4.81$, $r^2 = 0.999$). This supports the assertion that the oxygenation mechanism includes the rate-determining SET step, as shown in Scheme 1. The large $k_{ox}$ values compared with the corresponding $k_{et}$ values, with the exception of **1e** in the $k_{ox} - k_{et}$ correlation, may be ascribed to the possible contribution of an intermolecular charge-transfer interaction between $\mathbf{2}^-$ and $O_2$, which could accelerate the oxygenation reaction.[26,27]

The *Cypridina* luciferin model **1f** showed a $k_{obsd}$ value similar to that of the 4-(*N*,*N*-dimethylamino)phenyl derivative **1a**. The $E_{ox}^{1/2}$ value of $\mathbf{2f}^-$ is also similar to that of $\mathbf{2a}^-$. These results indicate that the electronic effects of the 3-indolyl group on the chemiluminescent reactivity of the imidazopyrazinone ring is comparable with that of the 4-(*N*,*N*-dimethylamino)phenyl group. Therefore, the 3-indolyl group in *Cypridina* luciferin acts as an electron-donating group and plays an important role to increase the efficiency of the oxygenation of $\mathbf{2}^-$ via the SET mechanism.

In conclusion, our experimental findings strongly suggest that the chemiluminescence reaction of imidazopyrazinone (**1**) proceeds in a mechanism including the rate-determining SET from $\mathbf{2}^-$ to $O_2$ (Scheme 1). The SET-oxygenation mechanism can explain the processes from $\mathbf{2}^-$ to $\mathbf{3}^-$, although direct evidence for the generation of $\mathbf{2}^{\bullet}$ and $O_2^{\bullet -}$ is not yet available. There is also a strong suggestion that the *Cypridina* bioluminescence reaction is governed by the SET-oxygenation mechanism, as predicted by Goto.[1] Therefore, the SET-oxygenation process may be regarded as the most likely mechanism for bioluminescence and chemiluminescence reactions, as has been reported for chemiluminescence reactions of several electron-rich compounds.[28–30] Further study to reveal the complete chemiluminescence reaction mechanism of imidazopyrazinones is now in progress in our lab.

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11. An intensity ($I$) of the total emitted light (400–700 nm) was monitored using a Hamamatsu R5929 photomultiplier tube powered by a Hamamatsu C4900 power supply. The signal from the photomultiplier was collected on a PC computer and the data were analyzed with the graphics program Igor Pro, Version 4. 0. 8. 0 (Wave Metrics, Inc.).
12. The pseudo-first-order rate constant ($k_{\mathrm{obsd}}$) was obtained by analysis using the equation: $\ln I_t = -k_{\mathrm{obsd}}t + \ln I_0$, where $I_0$ and $I_t$ are the intensities at $t = 0$ and $t$, respectively. The experimental errors of $k_{\mathrm{obsd}}$ were within ±10%.
13. Chemiluminescence spectra were recorded on an ATTO AB-1850 spectrometer. The $\Phi_{\mathrm{CL}}$ values were determined as quantum yields relative to the $\Phi_{\mathrm{CL}}$ (0.013) of luminol in DMSO containing *t*-BuOK/*t*-BuOH under air.[14] The experimental errors of $\Phi_{\mathrm{CL}}$ were within ±10%. The yields of **4** were determined by GC analyses.
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16. The $k_{\mathrm{ox}}$ value of 22 $\mathrm{mol}^{-1}$ L $s^{-1}$ for **1c** was determined with the same apparatus as in Note 15, while those shown in Table 1 were determined with the apparatus described in Note 11. The values for **1c** from the two apparatuses agreed within experimental error.
17. Chemiluminescence reactions of imidazopyrazinones **1** gave the corresponding amidopyrazines **6** as main products (73–100% yields). Observed $\lambda_{\mathrm{em}}$ for each **1** indicates that the light emission occurred from $\mathbf{5}^{-*}$.
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