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

Hiroyuki Kondo,[a] Takayuki Igarashi,[a] Shojiro Maki,[a] Haruki Niwa,[a]
Hiroshi Ikeda[b] and Takashi Hirano[a],*

[a]Department of Applied Physics and Chemistry, The University of Electro-Communications, Chofu, Tokyo 182-8585, Japan
[b]Department of Chemistry, Graduate School of Science, Tohoku University, Sendai 980-8578, Japan

Abstract—Kinetics of chemiluminescence reactions of 2-methyl-6-phenylimidazo[1,2-a]pyrazin-3(7H)-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(7H)-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 (**3⁻**) is suggested by Goto[1,5] in the chemiluminescence reaction of 2-methylimidazo-[1,2-a]pyrazin-3(7H)-one in diglyme, containing t-BuOK as a base. The process from **1** to **3⁻** is triggered by deprotonation of **1** with a base. The successive single electron transfer (SET) from the resulting **2⁻** to O_2 probably gives a radical **2·** and the superoxide anion $O_2^{·-}$. Fast radical coupling of **2·** and $O_2^{·-}$ followed by a rearrangement affords a dioxetanone intermediate (**4⁻**) via **3⁻**. Finally, thermal decomposition of **4⁻** with the loss of carbon dioxide generates a singlet-excited state of amidopyrazine anion (**5⁻***), which emits light, yielding 2-acetamide-5-arylpyrazine (**6**) via protonation of **5⁻**. The SET from **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-indolyl derivative (**1f**), a prototype model of Cypridina luciferin.[8] We report here that the oxygenation reaction of **2⁻** is controlled by SET from **2⁻** to O_2,
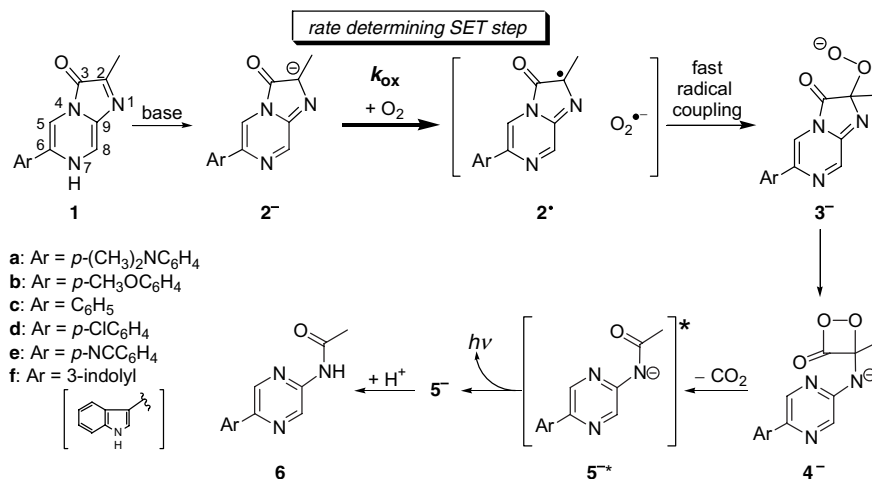
Scheme 1.

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** (1.0×10^{-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. 2.9×10^{-2} s^{-1}.[12] Interestingly, no significant dependency on the concentration of TMG ([TMG] = $1-100 \times 10^{-4}$ mol L^{-1}) was observed for (i) emission wavelengths from **5c**$^{-*}$ (λ_{em} = 467 nm), (ii) k_{obsd}, (iii) chemiluminescence quantum yields (Φ_{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.
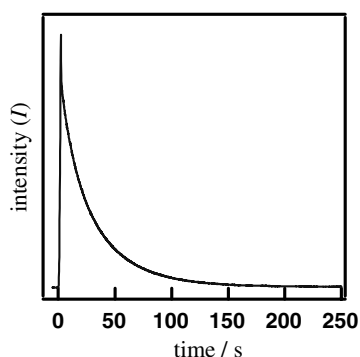
Next, we examined the chemiluminescence reactions of **1c** in the presence of O$_2$ at various concentrations.[10] The reactions of **1c** were similarly traced in acetonitrile containing TMG (0.10 mol L^{-1}) at 25 ± 1 °C.[15] A linear correlation between k_{obsd}[12] and [O$_2$], k_{obsd} = k_{ox}[O$_2$] + 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 λ_{em} and Φ_{CL}.[11–13,17] The log[k_{ox}(**1**)/k_{ox}(**1c**)] values are correlated to the Hammett σ_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_{ox}^p) 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_{ox}^p values of **2**$^-$. This tendency matches the result of the Hammett correlation of the log[k_{ox}(**1**)/k_{ox}(**1c**)] versus σ_p. Judging from

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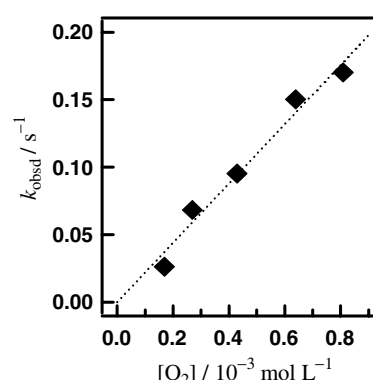

Figure 2. Correlation between the pseudo-first-order rate constants (k_{obsd}) for **1c** and concentrations of O$_2$ ([O$_2$]).