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Study of the characteristics of three high-energy intermediates generated in peroxyoxalate chemiluminescence (PO-CL) reactions

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Perylene emission intensity generated from peroxyoxalate chemiluminescence (PO-CL) reactions was studied as a function of time and order of reagent addition. Based on ^{1}H-NMR analyses, kinetics of UV absorbance and emission intensity *vs.* time profiles, we conclude that PO-CL reactions in the presence of imidazole (ImH) can proceed by three distinct reaction pathways depending on how the reagents are mixed together. When bis(2,4,6-trichlorophenyl) oxalate (TCPO) is mixed simultaneously with H_2O_2, ImH and perylene, a slowly decaying emission curve is generated from the interaction between perylene and a high-energy intermediate (*i.e.*, six- or eight-membered cyclic compound) formed by the ImH-catalyzed nucleophilic reaction (TCPO-CL reaction). Upon mixing TCPO simultaneously with ImH and perylene in the absence of H_2O_2, however, distinctly different CL curves of lower intensity are generated from the interaction between perylene and a new, unknown high-energy intermediate formed from the reaction between the aryl oxalate and ImH. Finally, using ^{1}H-NMR, we observed that 1,1′-oxalyldiimidazole (ODI) is also formed from the reaction between TCPO and ImH. When ODI reacts with excess H_2O_2 in the presence of perylene, a higher intensity and relatively fast decaying emission curve is generated (ODI-CL reaction) from the interaction between perylene and the high-energy intermediate produced, which we propose is imidazolylhydroxydioxetanone or 1,2-dioxetanedione.

Introduction

Peroxyoxalate chemiluminescence (PO-CL) generated from the reaction between aryl oxalates and H_2O_2 in the presence of a catalyst has been widely used to analyze various trace fluorophores because it is more sensitive than fluorescence and UV absorbance.[1–5]

Since Rauhut *et al.* first proposed 1,2-dioxetanedione as a high-energy intermediate capable of transferring energy to fluorophores in PO-CL reactions,[6] several authors have proposed structures and properties of other possible high-energy intermediates generated in these reactions.[7–20] Alvarez *et al.*[8] proposed that at least two high-energy intermediates are generated in PO-CL reactions, and several research groups[9–20] have subsequently proposed possible reaction pathways for these high-energy intermediates. These researchers generally used imidazole (ImH) to catalyze the PO-CL reaction because it is an efficient catalyst.[21]

Lee *et al.*[13] obtained both a fast decay curve and a slow decay curve from bis(2,4-dinitrophenyl) oxalate (DNPO) CL reactions in the presence of base catalysts (ImH or sodium salicylate). They proposed that the high-energy intermediate (1,2-dioxetanedione) producing the fast decay curve resulted from the acid–base reaction between the base catalyst and the hydroperoxyoxalate ester generated from the reaction between DNPO and H_2O_2. They also suggested that the intermediate (six-membered cyclic intermediate) generating the slow decay curve was formed by the ImH-catalyzed reaction proposed by Milofsky and Birks.[11] Both decay curves observed under their experimental conditions[13] were dependent on the concentrations of base catalyst and DNPO. However, in the CL intensity *vs.* time profile obtained from the bis(2,4,6-trichlorophenyl) oxalate (TCPO) CL reaction using a higher ImH concentration than that in the DNPO-CL reaction, only a slow decaying curve appeared. This is because the hydroperoxyoxalate ester generated from the reaction between TCPO and H_2O_2 has a higher pK_a than ImH.[13]

Neuvonen[14] suggested the formation of 1,1′-oxalyldiimidazole (ODI) as one of the intermediates formed in PO-CL reactions in the presence of ImH based on the ImH-catalyzed hydrolysis of bis(4-nitrophenyl) oxalate (4-NPO) and the observation of ImH-catalyzed hydrolysis of *p*-nitrophenyl acetate.[22] Hadd and Birks[17] proposed a nucleophilic substitution reaction of ImH with TCPO or DNPO from the UV absorbance of 2,4,6-trichlorophenol (TCP) or 2,4-dinitrophenol (DNP) produced in the reactions. They[19] also studied the ImH-catalyzed PO-CL reaction under both reaction conditions ([ImH], $[H_2O_2] \gg$ [TCPO] and [ImH] $\gg$ [TCPO] $\gg [H_2O_2]$) in the presence of 9,10-diphenylanthracene (DPA). They[19] proposed that ODI is formed from the reaction between TCPO and ImH in the presence of H_2O_2. Recently, Hadd *et al.*[23] proposed

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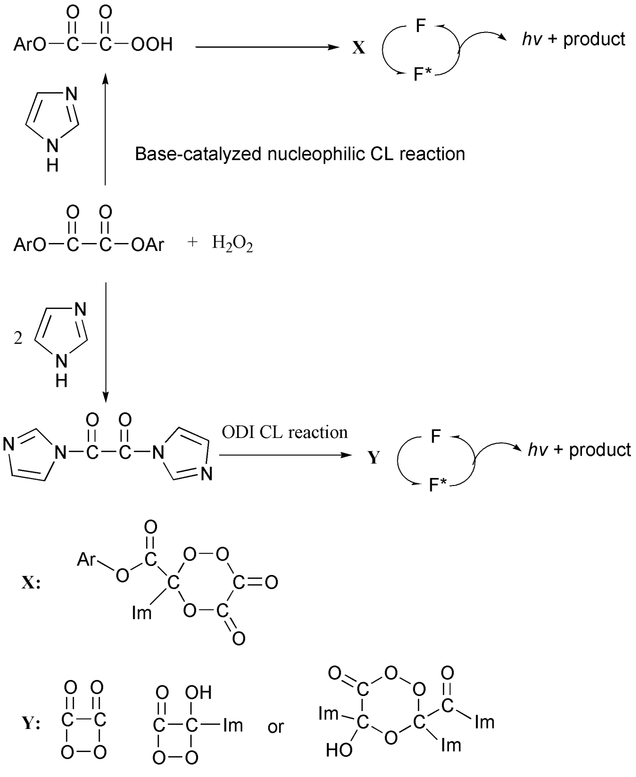
two possible types of intermediates formed in ODI-CL reactions based on the results observed from the reactions between ODI and various H_2O_2 concentrations in the presence of DPA.

Previous research groups[11–13,15,16,18–20,23] have studied ImH-catalyzed PO-CL reactions when the reagents were mixed simultaneously. As shown in Scheme 1, the base-catalyzed nucleophilic reaction proposed by two research groups[11,13] and the ODI-CL reaction suggested by other authors[12,15,16,18–20,23] have been used to explain observed CL decay curves. **X** and **Y** in Scheme 1 indicate high-energy intermediates proposed by previous research groups.[11–13,15,16,18–20,23]



Scheme 1

The motivation for this study was to gain additional information on the complex mechanisms and pathways of PO-CL reactions, particularly the characteristics of the high-energy intermediates generated by the reactions. In this research, we varied the addition of reagents to elucidate the properties of possible high-energy intermediate(s) formed in the presence of ImH. First, we mixed TCPO and the other reagents (ImH, H_2O_2 and perylene) simultaneously. Second, we mixed TCPO with ImH and allowed the mixture to react for a specified time before adding the mixture to a solution containing only perylene or H_2O_2 and perylene. The CL decay curve properties observed for the different reagent addition methods were distinctly different from one another. Based on these results, we propose possible reaction pathways for the three different systems.

2 Experimental

2.1 Chemicals

DNPO, TCPO, pentachlorophenyl oxalate (PCPO), TCP, H_2O_2 (50%), ODI (90%), perylene, and ImH were purchased from Aldrich. H_2O_2 was stored in a refrigerator at -10 °C and titrated with $KMnO_4$ to determine the exact concentration before use. Spectrophotometric grade ethyl acetate, acetonitrile, and dimethyl sulfoxide (DMSO) purchased from Baker were used as solvents. Deionized water of resistivity greater than 17.8 MΩ cm^{-1} was obtained from a Barnstead Nanopure system. Perdeuterated DMSO, acetonitrile, benzene, acetone, and chloroform were purchased from Cambridge Isotope Laboratories.

2.2 Chemiluminescence measurements

A typical CL reaction was conducted at room temperature (~22 to 23 °C) in a 1.0 cm fluorescence cell inserted in the sample compartment of a spectrofluorometer (PTI Inc.). Fresh solutions were prepared daily and kept in the dark. The CL intensity *vs.* time was monitored by the spectrofluorometer at the maximum emission wavelength (468 nm) of perylene without an illuminating light source. A small magnetic stir bar in the fluorescence cell provided continuous mixing during each experiment.

2.2.1 Simultaneous mixing of PO-CL reagents. A 0.5-ml H_2O_2 solution was added to the cell followed by addition of 0.5 ml each of a perylene solution and an ImH solution. The reaction was initiated by injecting 0.5 ml of an aryl oxalate (DNPO, TCPO or PCPO) solution, and each experimental condition was replicated three times.

The rate of TCP formation from the TCPO-CL reaction was followed by absorbance at 290 nm using the kinetics function of an 8452A diode array spectrophotometer (Hewlett Packard).

2.2.2 Mixing of perylene with ODI formed from the TCPO–ImH reaction in the absence and presence of H_2O_2. After mixing 0.5 ml of an aryl oxalate solution with 0.5 ml ImH solution in the cell for a specified time period, the CL reaction was initiated by injecting 1.0 ml of a perylene solution or a mixed perylene–H_2O_2 solution into the cell. Each experimental condition was replicated three times.

TCP generation from the reaction between TCPO and ImH in the absence of H_2O_2 was measured as described above.

2.3 NMR analyses

Samples of ODI were dissolved in d_6-DMSO, while TCPO, TCP and ImH were dissolved in d_3-acetonitrile or d_6-DMSO. ^{1}H-NMR spectra of ODI, TCPO, TCP, ImH, and solution mixtures of TCPO and ImH were recorded in flame sealed NMR tubes at ambient temperature with a UnityPlus 300 NMR Spectrometer. Spectra were obtained for various mixing times to determine if one of the products generated from the reaction between TCPO and ImH in DMSO or acetonitrile was ODI.

2.4 Kinetics analysis

CL intensity *vs.* time profiles obtained from the TCPO-CL and ODI-CL reactions were found to fit a simple two-step reaction scheme:

$$A \xrightarrow{k_r} B \xrightarrow{k_f} C \qquad (1)$$

where A and C represent reactants and products, respectively, and CL intensity is proportional to the concentration of intermediate species, B. Both steps in eqn. (1) are assumed to be irreversible, pseudo first-order reactions with rate constants k_r and k_f corresponding to the rise and fall, respectively, in the observed CL intensity *vs.* time profiles. The integrated rate equation then gives

$$\mathrm{CL} \propto [\mathrm{B}] = \frac{I_{\max}k_r}{k_f - k_r}[\exp(-k_r t) - \exp(-k_f t)] \qquad (2)$$

for the CL intensity *vs.* time.[24] Values for k_r, k_f and the maximum intensity, $I_{\max}$, for each CL time profile were obtained by nonlinear least-squares fitting of eqn. (2) to the experimental data using the optimization toolbox in Matlab 5.2. All reported rate constants are an average of at least three independent measurements.

3 Results and discussion

The CL intensity *vs.* time profile for simultaneous mixing of reagents ([TCPO] = 0.25 mM, [ImH] = 0.50 mM, [H_2O_2] = 50.0 mM, and [perylene] = 0.04 mM) in acetonitrile is shown in Fig. 1 (curve a). This reaction, which generated a relatively slow CL decay curve, will be referred to here as the TCPO-CL reaction. Fig. 1-b shows the curve obtained when mixing TCPO with ImH for 90 seconds before adding H_2O_2 and perylene; note that the reagent concentrations used for curve b were the same as those used for curve a. Curve b exhibits a biexponential decay profile having both a fast decaying component and a slowly decaying component.

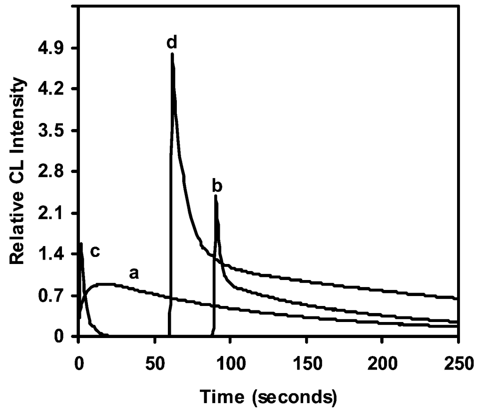


Fig. 1 CL intensity *vs.* time profiles in acetonitrile obtained under various experimental conditions (see text for details). (a) TCPO-CL reaction. (b) Addition of H_2O_2 and perylene after TCPO reaction with ImH for 90 seconds. (c) ODI-CL reaction. (d) Addition of H_2O_2 and perylene after mixing TCPO with ODI (~10% ImH impurity) for 60 seconds.

Fig. 1-c shows the CL intensity *vs.* time profile obtained for the ODI-CL reaction ([ODI] = 0.25 mM, [H_2O_2] = 50.0 mM, [perylene] = 0.04 mM). Several authors[12,14–20] have suggested that ODI would be generated from the reaction between ImH and TCPO. For this study, we used ODI purchased from Aldrich, which had a manufacturer-stated 10% (wt) impurity consisting primarily of ImH. Thus, upon mixing the ODI with TCPO (0.1 mM) for 60 seconds before adding H_2O_2 and perylene for the same ODI concentration used in Fig. 1-c, we obtained a biexponential decay curve (Fig. 1-d) having both a fast decaying component and a slowly decaying component, similar to that previously observed for curve b. Based on these results, it is reasonable to believe that the slow decay component in curves b and d is produced by the TCPO-CL reaction (*e.g.*, curve a) whereas the fast decay component of b and d is generated by the ODI-CL reaction (*e.g.*, curve c).

The maximum intensity, I_{max}, of curve d is 3.1 times larger than that of curve c. Upon mixing ODI with TCPO for 15 minutes under the otherwise same experimental conditions used for curve c, we obtained an I_{max} that was 16 times larger than that for curve c (data not shown). These results indicate that ODI is relatively unstable in acetonitrile,[25] with most of it decomposing to ImH before it can react with H_2O_2. Thus, the CL curve observed in Fig. 1-c resulted from the reaction between H_2O_2 and the remaining fraction of ODI.

3.1 UV and NMR analyses of the TCPO-CL and ODI-CL reactions

Fig. 2 shows TCP formation *vs.* time as measured by UV absorbance. The rate constant for TCP formation by the TCPO-CL reaction in the presence of excess H_2O_2 (0.050 ± 0.003 s^{-1}) is faster than that by the ODI-CL reaction (0.0060 ± 0.0008 s^{-1}). In other words, the reaction between TCPO and H_2O_2 in the presence of ImH (TCPO-CL reaction) is faster than that between TCPO and ImH (ODI-CL reaction).

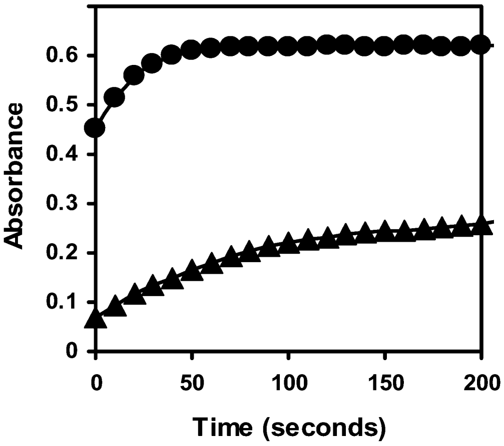


Fig. 2 UV absorbance (290 nm) of TCP *vs.* time for the reaction between 0.05 mM TCPO and 2.4 mM ImH in ethyl acetate in the presence (56.0 mM, ●) and absence (▲) of H_2O_2.

^{1}H-NMR spectra of ODI dissolved in d_6-DMSO revealed chemical shifts at 7.19, 7.78 and 8.43 ppm, whereas the chemical shifts for ImH and TCPO in d_6-DMSO were 7.00, 7.59 and 11.70 ppm, and 7.42 and 7.72 ppm, respectively. Although we could not detect the chemical shifts for ODI from the reaction of TCPO and ImH in the presence of H_2O_2 (TCPO-CL reaction), the chemical shifts for ODI from the reaction of TCPO and ImH without H_2O_2 (ODI-CL reaction) were observed. These results indicate that the high-energy intermediate formed from the TCPO-CL reaction differs from that produced in the ODI-CL reaction.

3.2 Effect of mixing time between TCPO and ImH

Fig. 3 shows the effect of reaction time between TCPO (0.05 mM) and ImH (2.4 mM) before adding H_2O_2 (56.0 mM) and perylene (0.05 mM) in ethyl acetate solutions. When all reagents were mixed simultaneously, a CL curve having a very long lifetime was observed. However, when the reaction time between TCPO and ImH prior to addition of H_2O_2 and perylene was increased, I_{max} of the CL curves initially increased

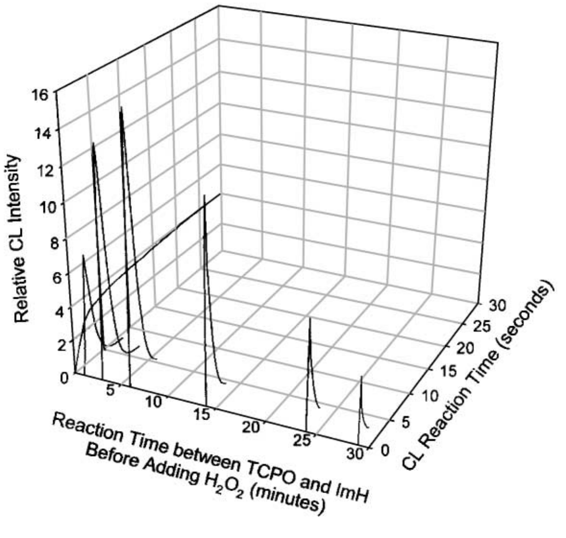


Fig. 3 CL intensity *vs.* time profiles obtained when TCPO was reacted with ImH for specified periods of time prior to addition of H_2O_2 and perylene in ethyl acetate solutions.

Table 1 Effect of ImH concentration in TCPO-CL reaction

[ImH]/mM	I_{max}	τ_{max}/s	τ_{half}/s	10^3k_r/s^{-1}	10^3k_f/s^{-1}
0.6	2.6	75.0	451	44.2 (0.6)	1.9 (0.04)
0.9	2.9	54.0	282	56.1 (0.3)	3.3 (0.01)
1.2	3.1	41.0	194	70.3 (0.4)	5.0 (0.02)
1.8	3.5	25.0	122	113.4 (0.3)	7.9 (0.03)
2.4	3.7	16.0	85	182.4 (0.4)	11.0 (0.03)

Table 2 Effect of H_2O_2 concentration in TCPO-CL reaction

[H_2O_2]/mM	I_{max}	τ_{max}/s	τ_{half}/s	$10k_r$/s^{-1}	10^3k_f/s^{-1}
1.8	0.51	18.0	102	1.82 (0.05)	7.98 (0.05)
7.2	0.59	17.0	98	1.90 (0.05)	8.21 (0.04)
28.0	0.71	16.0	91	2.12 (0.03)	9.02 (0.03)
56.0	0.78	14.5	84	2.39 (0.06)	10.31 (0.04)

for mixing times up to six minutes and then decreased exponentially with longer mixing times.

Because the ImH concentration was in excess over TCPO for the CL curves shown in Fig. 3, the slowly decaying component was not observed and thus did not complicate the analyses. It can be seen that as the reaction time between TCPO and ImH increased, the time to reach I_{max} (τ_{max}) and the half-life of the decay reaction, τ_{half}, each decreased for the CL curves. In the following sections, we report the effects of ImH, TCP and water, and discuss the differences between the TCPO-CL and ODI-CL reactions.

3.3 TCPO-CL reaction

3.3.1 Effects of ImH concentration. The CL intensity *vs.* time profile exhibiting a slowly decaying component (Fig. 1-a) was obtained by simultaneous mixing of the TCPO-CL reagents. Using this same experimental procedure, we determined the effect of ImH concentration for TCPO, H_2O_2 and perylene concentrations of 0.05, 56.0, and 0.05 mM, respectively, in ethyl acetate solutions (Table 1). As can be seen, I_{max} increased whereas τ_{max} and τ_{half} both decreased with increasing ImH concentration. In addition, the two pseudo first-order rate constants k_r and k_f exhibited second- and first-order dependences, respectively, on the concentration of ImH.

3.3.2 Effect of H_2O_2 concentration. Table 2 shows the effect of varying H_2O_2 concentration on I_{max}, τ_{max} and τ_{half} for ImH, perylene and TCPO concentrations of 3.0, 0.01 and 0.06 mM, respectively. As can be seen, I_{max} increased while τ_{max} and τ_{half} both decreased slightly with increasing H_2O_2 concentration. In addition, values for k_r and k_f also showed a slight increase with increasing H_2O_2 concentration.

3.3.3 Effect of water content. Several research groups[26–28] have observed that DNPO undergoes hydrolysis in solvents with a low water content, forming 2 moles of the corresponding DNP from 1 mole of DNPO. Based on this information, we hypothesized that TCPO might also undergo decomposition to TCP and carbon monoxide in solvents containing water even though TCPO is much more stable than DNPO.

As shown in Table 3, τ_{max} and τ_{half} decreased with increasing water content in the TCPO-CL system, while I_{max} first increased and then at a certain water content also began to decrease. We also investigated the effect of TCP (0.005 mM) for the same reaction conditions as Table 3 in the absence of water (data not shown). Upon adding TCP, I_{max} increased while τ_{max} and τ_{half} decreased slightly. Based on these results, we propose that the TCP generated from TCPO hydrolysis acts as a catalyst or nucleophile to enhance I_{max} and reduce τ_{max} and τ_{half} below a certain concentration but then acts as an inhibitor in the TCPO-CL reaction above this concentration. We also studied the effects of adding various other phenols (*e.g.*, 2,4-dichlorophenol, pentachlorophenol, DNP) which are final products in PO-CL systems on the TCPO-CL reaction and observed that I_{max}, τ_{max} and τ_{half} each showed a dependence on the pK_a of the phenols (data not shown).

Table 3 Effect of water content (% volume) in TCPO-CL reaction

[H_2O] (%)	I_{max}	τ_{max}/s	τ_{half}/s
0.0	0.22	52.0	298.0
4.0	0.43	39.0	205.0
8.0	0.48	31.0	187.0
12.0	0.52	24.0	134.0
20.0	0.39	18.0	85.0

[a] [TCPO] = 0.05 mM, [H_2O_2] = 1.80 mM, [ImH] = 1.0 mM, [perylene] = 0.05 mM, solvent: ethyl acetate.

3.3.4 Possible reaction mechanism. When the TCPO-CL reagents are mixed simultaneously, the first intermediate most likely to appear would be the hydroperoxyoxalate ester (ArCOCOOOH) because the reaction rate between TCPO and H_2O_2 is faster than that between TCPO and ImH (Fig. 2, Tables 1 and 2). Stevani *et al.*[15] suggested that 4-chlorophenyl hydrogen oxalate, one of the hydroperoxyoxalate esters, is stable in the presence of fluorescent compounds before adding weak bases such as ImH and *p*-chlorophenolate. Thus, 2,4,6-trichlorophenyl hydrogen oxalate, another hydroperoxyoxalate ester, might not be a high-energy intermediate capable of generating excited singlet states of fluorescent molecules.

Alvarez *et al.*[8] observed two peaks having different decay times in TCPO-CL reactions using triethylamine (pK_a 10.7 in water) as a weak base. Lee *et al.*[13] have studied the possible reaction mechanism for two CL decay curves generated from DNPO-CL reactions in the presence of various weak bases such as ImH and sodium salicylate, and have proposed that the fast decay curve observed by Alvarez *et al.*[8] is generated from the acid–base reaction while the slow decay curve is produced from the base-catalyzed nucleophilic reaction proposed by Milofsky and Birks.[11]

As shown in Fig. 1-a, when ImH (pK_a 6.95 in water) was used as a weak base instead of triethylamine, we did not observe a fast decay curve generated from the acid–base reaction because the pK_a of 2,4,6-trichlorophenyl hydrogen oxalate is higher than that of ImH.[13] In conclusion, the slow decay curve observed in this system is generated from the interaction between perylene and a high-energy intermediate (*i.e.*, an eight- or six-membered cyclic intermediate) formed by the ImH-catalyzed nucleophilic reaction.

3.4 Chemiluminescence generated from reactions between aryl oxalates and ImH in the absence of H_2O_2

We observed distinctly different CL intensity *vs.* time profiles when different aryl oxalates were mixed simultaneously with ImH and perylene in the absence of H_2O_2. Fig. 4 shows that the reactivity of the intermediate formed by this reaction depends on the properties of the substituted leaving group in a particular aryl oxalate. When perylene was mixed directly with TCPO or ODI in the absence of H_2O_2, only noise was detected. Undoubtedly, the profiles shown in Fig. 4 are generated from the interaction between perylene and a new high-energy intermediate formed from the reaction between aryl oxalates and ImH. Fig. 4 implies that the nucleophilic reaction of ester with ImH is more complicated than previously known.

3.4.1 NMR analysis. Fig. 5 shows ^{1}H-NMR spectra for TCPO and ImH in acetonitrile for different mixing times. Although five specific chemical shifts (7.19, 7.72, 7.80, 8.40 and 8.53 ppm) appeared between 2 and 10 min of mixing time, only three (7.19, 7.72 and 8.40 ppm) of them, all consistent with ODI, were observed at 30 min. The chemical shift for ODI

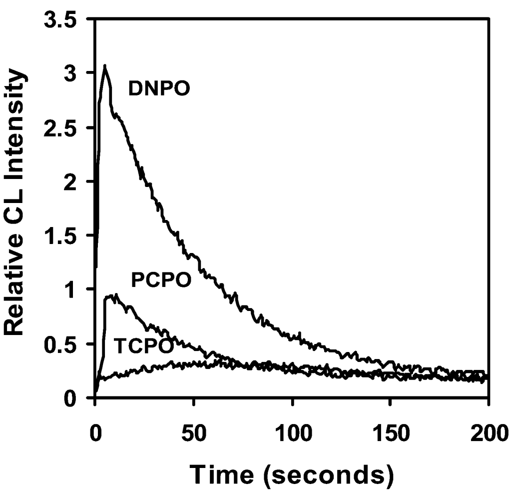


Fig. 4 CL generated from the reaction between ImH and three types of aryl oxalates in the presence of perylene without added H_2O_2; [oxalate] = 0.2 mM, [ImH] = 2.0 mM, [perylene] = 0.05 mM.

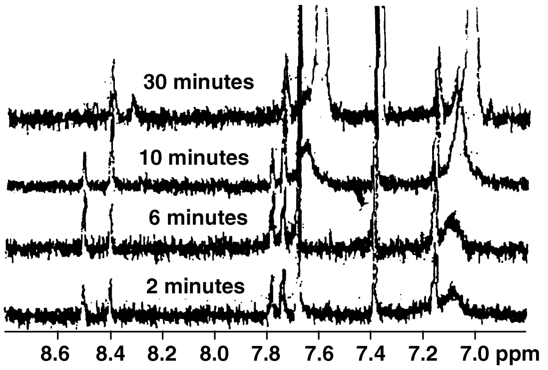
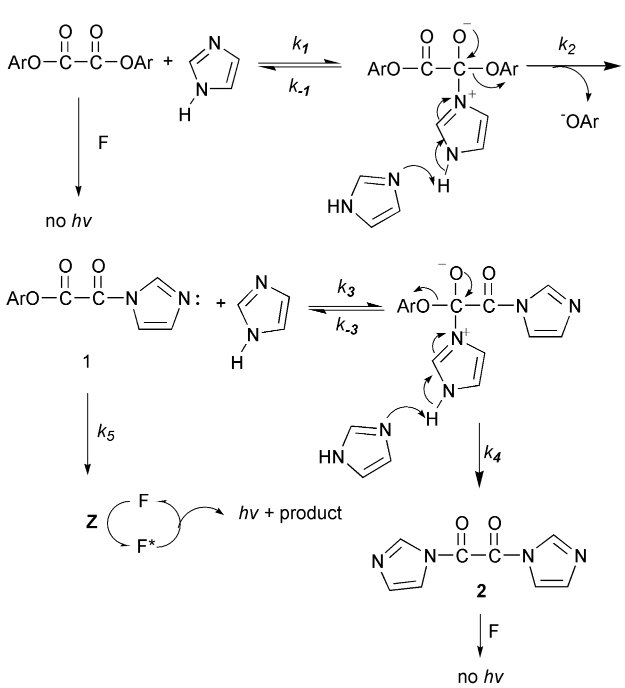


Fig. 5 ^{1}H-NMR spectra obtained after mixing TCPO with ImH for a specified time in acetonitrile.

Table 4 Effects of ImH concentration on k_r and k_f

[ImH]/mM	$10k_r$/s^{-1}	10^2k_f/s^{-1}
0.50	1.01 (0.08)	0.05 (0.006)
0.75	1.54 (0.06)	0.12 (0.012)
1.00	2.33 (0.15)	0.17 (0.021)
1.50	3.53 (0.09)	0.42 (0.023)
2.00	4.52 (0.12)	0.65 (0.019)

[a] [DNPO] = 0.1 mM, [perylene] = 0.05 mM, solvent: ethyl acetate.



Scheme 2

observed at 7.19 ppm was higher than the other two chemical shifts (7.72 and 8.40 ppm) between 2 and 10 min of mixing time, although the height ratio of the chemical shifts after 30 min was constant. This result indicates that the chemical shifts of the transient intermediate formed from the reaction between TCPO and ImH before ODI was produced not only appeared at 7.80 and 8.53 ppm but also at 7.19 ppm (*i.e.*, the chemical shift of the transient intermediate overlapped with that of ODI at 7.19 ppm).

3.4.2 Possible mechanism. Neuvonen[14] obtained UV spectra of a transient intermediate for the reaction of ImH with bis(4-nitrophenyl) oxalate and reported that the decomposition rate of the transient intermediate depended on the ImH concentration. Thus, the three chemical shifts (7.19, 7.80 and 8.53 ppm) observed in Fig. 5 could conceivably be the oxalyl derivative ($ImCOCO_2Ar$) proposed by Neuvonen.[22] However, the oxalyl derivative cannot explain the results shown in Fig. 4 because it does not generate singlet excited states in fluorophores by the chemically initiated electron exchange luminescence (CIEEL) mechanism.[29] Thus, we conclude that a new high-energy intermediate formed from the reaction between TCPO and ImH in the absence of H_2O_2 was not detected by the ^{1}H-NMR spectrometer, most likely because of its very short lifetime, which would be consistent with other high-energy intermediates formed in PO-CL reactions. Figs. 4 and 5 together indicate that the new high-energy intermediate can be formed from an oxalyl derivative before producing ODI, as demonstrated in Scheme 2. Unfortunately, we were unable to determine the identity of this new high-energy intermediate based on our results. The high-energy intermediate, **Z**, shown here with an undefined structure, is generated from **1**. We propose that **Z** is very unstable and has never been isolated, again as often is the case for other high-energy intermediates formed in PO-CL reactions.

Rising rate constants, k_r, and falling rate constants, k_f, were obtained from the pseudo-first-order reaction[8] (Table 4). A first-order dependence of k_r on the ImH concentration was observed, whereas a second-order dependence resulted for k_f. k_r, necessary to form **1** in the reaction between DNPO and ImH, illustrates the first-order dependence on ImH concentration because the reaction of the tetrahedral intermediate that forms **1**, k_2[ImH], is faster than the reverse reaction, k_{-1}. k_f, necessary to form **Z** and **2** in the reaction between **1** and ImH, shows a second-order dependence on ImH concentration. This is because the pathway for forming **2** is predominant compared to that for generating **Z**. Also, the base-catalyzed breakdown of the zwitterionic addition intermediate is the rate-limiting step of this reaction in organic solvents ($k_{-3} \gg k_4$ [ImH]).[14,17,22,30] k_f is approximately equal to k_4K_a[DNPO][ImH]^2 where $K_a = k_3/k_{-3}$.

3.5 ODI-CL reaction

As shown in Fig. 1, the CL decay curve obtained from the ODI-CL reaction is faster than that observed in the TCPO-CL reaction. The CL decay curve observed in the ODI-CL reaction is generated by the interaction between perylene and a high-energy intermediate formed from ODI and H_2O_2.

3.5.1 Effect of ImH concentration. We observed CL intensity *vs.* time profiles for varying ImH concentration (0.6 to 2.4 mM) using the method of Fig. 3 to understand the details of the reaction between TCPO and ImH that forms ODI. I_{max} shown in Fig. 6 indicates the maximum of the CL intensity *vs.*

Table 5 Effect of ImH concentration on the reactions between TCPO and ImH before adding H_2O_2 and perylene in ODI-CL reactions

[ImH]/mM	I_{max}	τ_{max}/s	τ_{half}/s	10^3k_r/s^{-1}	10^3k_f/s^{-1}
0.6	18.9	~1800	~5220	0.38 (0.03)	0.82 (0.03)
0.9	31.0	~1260	~3420	0.72 (0.04)	0.87 (0.04)
1.2	25.3	~840	~2400	1.30 (0.04)	0.95 (0.03)
1.8	19.7	~540	~1740	2.77 (0.05)	1.05 (0.03)
2.4	15.6	~360	~1140	5.33 (0.03)	1.17 (0.03)

[a] Reaction conditions are shown in Fig. 6.

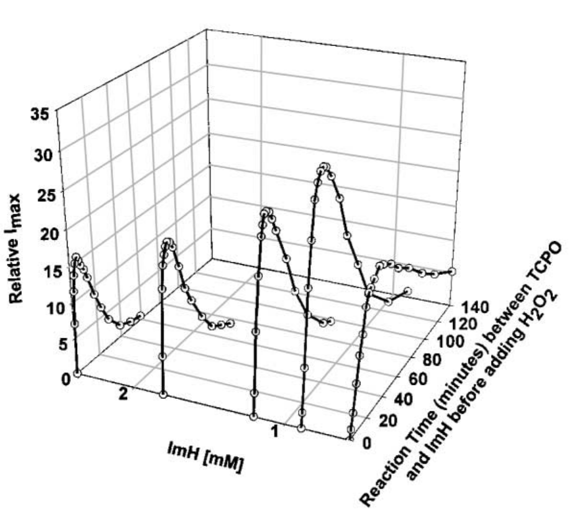


Fig. 6 Effect of ImH concentration on the reaction between TCPO and ImH before adding H_2O_2 and perylene in ODI-CL reactions in ethyl acetate. [H_2O_2] = 56.0 mM, [TCPO] = 0.05 mM, [perylene] = 0.05 mM.

Table 6 Effect of water content (% volume) in ODI-CL reactions

[H_2O] (%)	0	8.0	12.0	16.0	20.0
Relative I_{max}	0.64	2.26	3.99	1.42	0.43
STD	0.03	0.12	0.21	0.15	0.05

[a] Reaction conditions: [TCPO] = 0.05 mM, [ImH] = 0.5 mM, [H_2O_2] = 20.0 mM, [perylene] = 0.03 mM, TCPO mixed with ImH for 120 s before adding H_2O_2 and perylene.

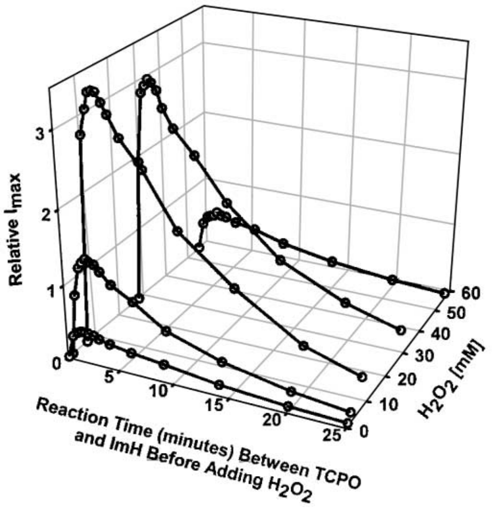


Fig. 7 Effect of H_2O_2 concentration on the reactions between TCPO and ImH before adding H_2O_2 in ODI-CL reactions at 22–23 °C. [TCPO] = 0.03 mM, [ImH] = 5.0 mM, [perylene] = 0.05 mM.

time profile observed for each experimental condition. As can be seen, I_{max} increased up to a certain point and then decreased exponentially with an increase in the mixing time for the reaction between TCPO (0.05 mM) and each ImH concentration before adding perylene (0.05 mM) and H_2O_2 (56.0 mM) in ethyl acetate. τ_{max} and τ_{half} for the data presented in Fig. 6 decreased as the ImH concentration increased. This is because the excess ImH used in the ODI-CL reaction catalyzes the breakdown of ODI formed from the reaction between TCPO and ImH. Neuvonen[14] and Stigbrand *et al.*[25] have suggested that the decomposition rate of ODI is dependent on the concentration of ImH.

Several research groups[14,16,17] have obtained rate constants for TCP production from the reaction between TCPO and ImH using a pseudo-first-order reaction method and then used UV absorbance of TCP to predict indirectly the rate constants of ODI formation in their systems. With the CL intensity *vs.* time profiles shown in Fig. 6, we were able to directly determine the rate constants of ODI production from the reaction between TCPO and ImH (Table 5). We are unaware of any prior use of this method. The upward curvature of the trend for k_r obtained from this new kinetics method indicates a second-order dependence on the ImH concentration, a result consistent with those obtained by Neuvonen[14] and Hadd and Birks.[17] The fall rate constant, k_f, exhibits a first-order dependence on the ImH concentration. We interpret this result to mean that the decomposition of ODI is accelerated with increasing ImH concentration, an interpretation consistent with results observed by Stigbrand.[25]

3.5.2 Effect of H_2O_2 concentration.

Fig. 7 shows the effect of H_2O_2 concentration on I_{max} determined when H_2O_2 and perylene were added to the solution after premixing TCPO and ImH in ethyl acetate for various times similarly to Figs. 3 and 6. I_{max} first increased and then began to decrease with increasing H_2O_2 concentration because ODI formed by the reaction between TCPO and ImH reacts with H_2O_2 to produce a high-energy intermediate. However, k_r (0.031 ± 0.003 s^{-1}), k_f (0.0015 ± 0.0002 s^{-1}), τ_{max}, and τ_{half} for the profiles shown in Fig. 7 were independent of H_2O_2 concentration under these experimental conditions because H_2O_2 reacts with ODI.

3.5.3 Effect of water content.

Table 6 shows the effect of water content in the ODI-CL reaction solution. As the water content increased, I_{max} initially increased and then began to decrease above 16%. In particular, I_{max} obtained in the solvent containing 12% water was approximately 6.25 times higher than that observed in acetonitrile alone. The decay time of the CL intensity *vs.* time profile measured with water present was faster than that observed without water, a result consistent with that reported by Stigbrand *et al.*[25] Under our experimental conditions, the reaction rate between TCPO and ImH in aqueous solvent would be faster than that in a purely nonaqueous solvent because water is widely known to accelerate PO-CL reactions.[6,31,32] In other words, the reaction kinetics between TCPO and ImH depends on the concentration of water in ODI-CL reaction systems. The concentration of ODI generated by the 120-s reaction between TCPO and ImH in the organic solvent containing 12% water is likely the highest in our experiments. Thus, when TCPO reacts with ImH for 120 s in an aqueous solvent containing 16 or 20% water, the ODI formed is undergoing a fast decomposition during that reaction time. If this hypothesis is correct, the reaction time between TCPO and ImH would have to be reduced to measure higher I_{max} values for the ODI-CL system using a solvent composed of 20% : 80% (v/v) water and acetonitrile.

The results shown in Table 6 likely are observed because of the formation of TCP from TCPO hydrolysis. To determine the effect of TCP concentration in the ODI-CL reaction, we added TCP (0.01 to 0.05 mM) to the mixture of TCPO (0.5 mM) and

ImH (5.0 mM) in dried acetonitrile and waited 2 minutes before adding H_2O_2 (20.0 mM) and perylene (0.05 mM). I_{max} observed under these reaction conditions was equal to or slightly lower than those obtained for ODI-CL reactions without added TCP We conclude that I_{max} obtained from the ODI-CL reaction is not enhanced by adding TCP even though I_{max} observed from the TCPO-CL reaction is extremely dependent on TCP concentration.

3.5.4 Possible reaction mechanism. Using ^{1}H-NMR spectroscopy, we confirmed the formation of ODI by the reaction between TCPO and ImH in the absence of H_2O_2. As shown in Table 5, when TCPO reacts with excess ImH to form ODI the upward trend for k_r indicates a second-order dependence on the ImH concentration, consistent with the results reported for reactions of esters with ImH.[27]

Orosz[33] investigated the hydrolysis of various diaryl oxalates using organic solvent–water CL systems and gas chromatography. Neuvonen[14] also measured rate constants for the hydrolysis of 4-NPO using UV absorbance. Both researchers postulated that the release of the first phenol group for each oxalate is much faster than that of the second one. Neuvonen[14] also proposed that the reaction step leading to the formation of the oxalyl derivative by the reaction between NPO and ImH is fast compared with the second reaction step to form ODI. However, results obtained by other research groups[16,17] for the reaction between TCPO and ImH in the absence of H_2O_2 are in contrast with those of Neuvonen.[14] For example, Hadd and Birks[17] have suggested that substitution of ImH for one TCP in TCPO will be slower than that of ImH for the second substitution because of the order of activation by the acyl groups toward substitution on the adjacent carbonyl (Im(C=O) > TCP(C=O)). We also discussed the detailed mechanism to form ODI for the reaction between TCPO and ImH in the previous section of this paper. Based on the references and our results obtained in Section **3.4**, k_r values shown in Table 5 are related to the nucleophilic reaction of ImH toward TCPO molecules.

ODI formed from the reaction between TCPO and ImH in the absence of H_2O_2 subsequently reacts with added H_2O_2 to produce a high-energy intermediate capable of generating excited singlet states of perylene. We investigated the effect of H_2O_2 concentration added to the solution after the reaction between TCPO and ImH in ethyl acetate for 60 s (Fig. 8). The fast decaying CL curves generated by the ODI-CL reaction are more sensitive to varying H_2O_2 concentration than are the slowly decaying CL curves produced by the TCPO-CL reaction over a wide range of concentrations. To determine the effect of H_2O_2 concentration in detail, H_2O_2 and perylene were added to solutions after TCPO had reacted with excess ImH in ethyl acetate for 240 s. The slowly decaying CL curve disappeared.

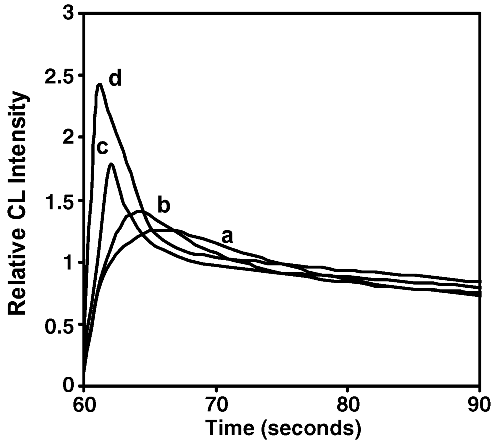


Fig. 8 Effect of H_2O_2 concentration on ODI-CL reactions (fast decaying curves) and TCPO-CL reactions (slowly decaying curves) after TCPO reacted with ImH in ethyl acetate for 60 s. [TCPO] = 0.25 mM, [ImH] = 0.5 mM, [perylene] = 0.05 mM. [H_2O_2] (mM) = (a) 2.0, (b) 7.0, (c) 16, and (d) 56.

However, we were not able to show an effect of H_2O_2 concentration and are not yet able to propose a high-energy intermediate formed by ODI-CL reaction. This is because the τ_{max} and τ_{half} of the CL decay curves were too short to calculate the rate constants (k_r and k_f) for each peak by the presumed pseudo-first-order reaction. The high-energy intermediate generated by the ODI-CL reaction might come from imidazolyl peracid (ImCOCOOOH), analogous to hydroperoxyoxalate ester (ArCOCOOOH) formed in the first reaction step of mechanisms proposed for PO-CL reactions. We showed that I_{max}, τ_{max} and τ_{half} in ODI-CL reactions decrease with increasing H_2O_2 concentration in Fig. 8. This is because H_2O_2 substitution for ImH of imidazolyl peracid to form dihydroperoxyoxalate (HOOCCOCOOOH), known to be a low-energy intermediate, occurs competitively with the reaction to form high-energy intermediate(s) from imidazolyl peracid when ODI reacts with excess H_2O_2. Based on the results obtained, the most plausible structures of high energy intermediate(s) formed from the intramolecular cyclization of imidazolyl peracid may be imidazolylhydroxydioxetanone or 1,2-dioxetanedione because of the relatively excess H_2O_2 added in our system. As Hadd *et al.*[23] suggested recently, however, when H_2O_2 concentrations much lower than those of TCPO and ImH are added in the ODI-CL reaction system, the possible structure of the high-energy intermediate (ImH-substituted six-membered cyclic intermediate) may be similar to that suggested for the TCPO-CL reaction in Section **3.3** of this paper.

4 Conclusions

In this paper, we present evidence for three distinct PO-CL reaction pathways catalyzed by ImH. By comparing UV absorbances, we show that the reaction between TCPO and H_2O_2 to form hydroperoxyoxalate ester is much faster than that between TCPO and ImH to form ODI. When all TCPO-CL reagents are mixed simultaneously (TCPO-CL reaction), the observed CL intensity *vs.* time profiles consistently generate relatively long decay curves for a wide range of H_2O_2 concentrations. We propose that the high-energy intermediate formed in TCPO-CL reactions is the same six- or eight-membered cyclic intermediate proposed by Milofsky and Birks[11] and Lee *et al.*[13] We also observe relatively low CL intensities when aryl oxalates are mixed simultaneously with ImH and perylene in the absence of H_2O_2. Unfortunately, the high-energy intermediate generated under these conditions could not be resolved based on our experimental results. Finally, we confirmed the formation of ODI from the reaction between TCPO and ImH using ^{1}H-NMR. When TCPO reacts with ImH in the presence of perylene before the other CL reagents are added to the solution, a relatively fast decaying curve appears under excess H_2O_2 conditions. Based on these results, the ODI-CL reaction pathway appears to differ from the pathways reported by Alvarez *et al.*[8] and Lee *et al.*[13] However, the possible structures of high-energy intermediates formed in the ODI-CL reaction may be analogous to those suggested for PO-CL. Table 7 summarizes the differences between TCPO-CL and ODI-CL reactions.

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Table 7 Comparison between the TCPO-CL reaction and the ODI-CL reaction in the presence of ImH

	TCPO-CL reaction	ODI-CL reaction
τ_{max}	Slow	Very fast
Sensitivity	Low	High
H_2O_2	CL intensity increases and τ_{max} and τ_{half} decrease with its increase.	CL intensity increases to a certain point and then decreases and τ_{max} and τ_{half} decrease with its increase.
ImH	CL intensity increases and τ_{max} and τ_{half} decrease with its increase.	CL intensity increases to a certain point and then decreases with its increase.
H_2O	CL intensity increases with its addition to a certain point and then begins to decrease.	CL intensity increases with its addition to a certain point and then begins to decrease.

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