Fast peroxyoxalate chemiluminescence for minimized analytical separation systems

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The maximum intensity, I_{max}, and time required to reach the maximum emission, τ_{max}, for 1-aminopyrene monitored in 1,1′-oxalyldi-4-methylimidazole (OD4MI) chemiluminescence (CL) reactions are approximately 61 times higher and 16 times faster than their respective values for bis(2,4,6-trichlorophenyl)oxalate (TCPO) CL reactions in the presence of imidazole (ImH).

Recently, we[1] reported evidence for two distinct peroxyoxalate chemiluminescence (PO-CL) reaction pathways (Scheme 1). These alternative pathways, which we termed the bis(2,4,6-trichlorophenyl)oxalate (TCPO)-CL and 1,1′-oxalyldiimidazole (ODI)-CL reactions, were dependent on the mixing order of the CL reagents. We also suggested that method development for analyzing polycyclic aromatic hydrocarbons (PAHs) under ODI-CL reaction conditions might be better than under TCPO-CL reaction conditions because the CL curve generated was much stronger and faster.[1]

In this paper, we have studied the effects of three imidazole derivatives (ImH, 2-methylimidazole (2-MImH), and 4-methylimidazole (4-MImH)) in TCPO-CL reactions. In addition, the characteristics of ODI, 1,1′-oxalyldi-2-methylimidazole (OD2MI) and 1,1′-oxalyldi-4-methylimidazole (OD4MI) formed from reactions between TCPO and the corresponding imidazole derivatives prior to addition of H_2O_2 and luminophore were investigated for the PO-CL reaction pathway shown in the bottom half of Scheme 1. Characteristics of the intermediates formed under each CL reaction condition will contribute to a more detailed understanding of PO-CL mechanisms. We selected 1-aminopyrene as the chemiluminophore because amino- and nitro-PAHs are important environmental contaminants that are generally more toxic (*e.g.*, mutagenic, carcinogenic) than their respective PAH parent compounds.[2,3]
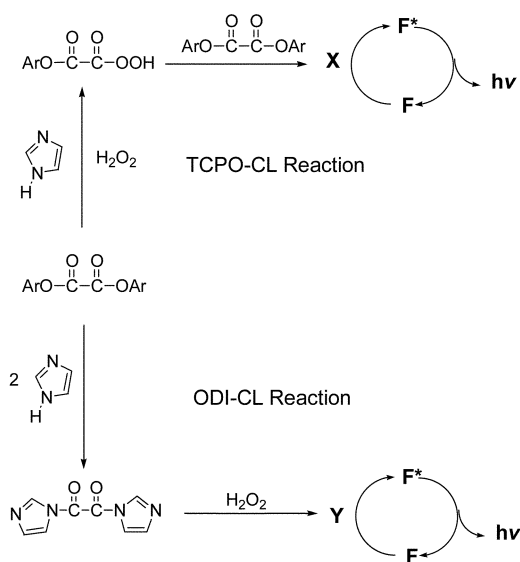
Because fluorescence quantum yields of nitro-PAHs are generally small, they are often reduced to amino-PAHs before their analysis using fluorescence[4] and chemiluminescence[5] techniques. Therefore, the results presented in this paper would be useful for developing improved methods to determine low-level concentrations of amino- and nitro-PAHs.

Table 1 shows that the maximum intensity, I_{max}, time to reach the maximum emission, τ_{max}, and the half-life of the decay reaction, τ_{half}, are dependent on the properties of the ImH derivatives used in TCPO-CL reactions. τ_{max} and τ_{half} for 2-MImH were much faster than their respective values for 4-MImH and ImH because the pK_a of 2-MImH is greater. However, I_{max} for 2-MImH was only slightly higher than that for 4-MImH, although both ImH derivatives generated I_{max} values approximately four times higher than ImH itself. This is because formation of the 2-MImH-substituted six-membered cyclic intermediate (**X-2**) generated in the TCPO-CL reaction is more sterically hindered than is the formation of the 4-MImH-substituted ring (**X-3**).

As shown in Fig. 1, UV absorbance of 2,4,6-trichlorophenol (TCP) monitored at 290 nm *vs.* time for the reaction between TCPO and 4-MImH indicated that the formation of OD4MI was much faster than formation of ODI and OD2MI because of the better nucleophilicity of 4-MImH (4-MImH > ImH > 2-MImH). The formation of OD2MI from the reaction between TCPO and 2-MImH was much slower because of the steric hindrance of 2-MImH.

The concentration of each ODI derivative shown in Table 2 depends on the reaction time between TCPO and the corresponding ImH derivative. The highest I_{max} was observed when OD4MI, formed from the reaction between 4-MImH and TCPO for 45 seconds, reacted with H_2O_2 in the presence of 1-aminopyrene (OD4MI-CL reaction). At mixing times longer than 45 seconds for TCPO and 4MImH in the absence of H_2O_2 and 1-aminopyrene, I_{max} decreased exponentially. This is because the excess 4-MImH used in the reaction catalyzed the

Scheme 1

Table 1 Effect of ImH derivatives in TCPO-CL reactions

ImH derivative	pK_a[6]	I_{max}[a]	τ_{max} (s)	τ_{half} (s)
ImH	6.99	1.0	7.9	109.7
2MImH	7.85	4.0	0.9	18.7
4MImH	7.55	3.7	3.3	29.1

[a] Relative I_{max} for each experimental condition was normalyzed by that observed for the TCPO-CL reaction in the presence of ImH. Reaction condition: [TCPO] = 0.1 mM, [H_2O_2] = 10.0 mM, [catalyst] = 2.0 mM, [1-aminopyrene] = 0.3 mM, solvent : ethyl acetate.
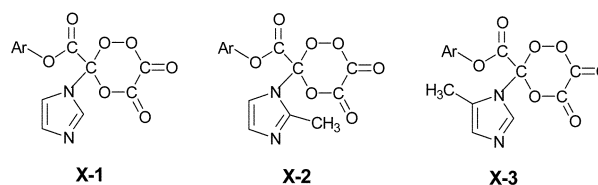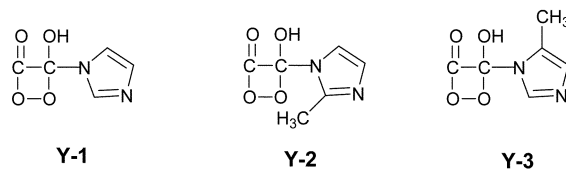
X-1   X-2   X-3

decomposition of OD4MI formed from the reaction between TCPO and 4-MImH.[1,7,8] In addition, I_{max} of 1-aminopyrene in the OD4MI-CL reaction was much higher than the corresponding values for the ODI- and OD2MI-CL reactions. Even though τ_{max} values were similar for all ODI derivatives, τ_{half} values were clearly different, particularly for the OD2MI-CL reaction. In conclusion, the results shown in Table 2 indicate that the characteristics of high-energy intermediates (**Y**) capable of transferring energy to 1-aminopyrene by the chemically initiated electron-exchange luminescence (CIEEL) mechanism[9] in Scheme 1 are dependent on the properties of the reactants (ODI, OD2MI, and OD4MI). In other words, the plausible high-energy intermediate structure (**Y-3:** 4-methylimidazolehydroperoxydioxatanone) formed from the reaction between OD4MI and H_2O_2 is slightly different from those (**Y-1:** imidazolehydroxydioxetanone or **Y-2:** 2-methylimidazolehy-

Y-1   **Y-2**   **Y-3**

droperoxydioxatanone) produced from the reaction between ODI (or OD2MI) and H_2O_2.

Many of the minimized analytical separation systems being developed today such as capillary electrophoresis[10] and microchips[11] require observation times in the millisecond range to preserve the band resolution. Unfortunately, the kinetics of the PO-CL reaction pathway (the upper reaction pathway in Scheme 1) selected by most research groups to determine detection limits of luminophores capable of accepting energy from **X-1** are so slow that extra flow elements are needed to observe the reaction in a time window at maximum emission intensity. An analytical separation system with extra flow elements would generally be larger than a corresponding minimized system but not have better resolution due to band broadening. As shown in Tables 1 and 2, values for I_{max} and τ_{max} observed with 1-aminopyrene and **Y-3** are 61.1 times larger and 15.8 times faster than their respective values obtained from the interaction between 1-aminopyrene and **X-1**. These results suggest that advanced analytical methods to determine low-level concentrations of amino- and nitro-PAH can be developed using the former reaction pathway (the bottom reaction pathway in Scheme 1). Also, the preliminary experimental results shown in this paper suggest that advanced microanalytical separation systems with PO-CL detection could be developed to determine low-level concentrations of luminophores.

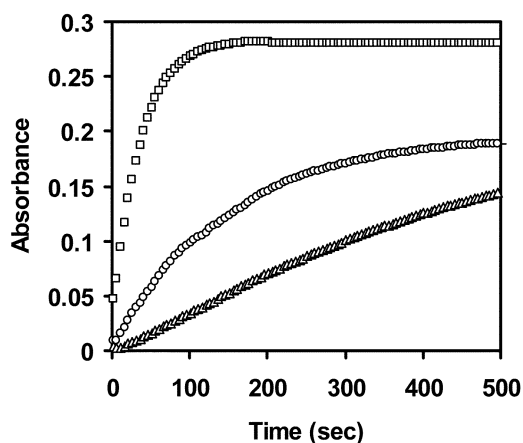**Fig. 1** UV absorbance (290 nm) of TCP *vs*. time for the reaction between 0.02 mM TCPO and 1.0 mM ImH derivative (□: 4MImH, ○: ImH, and △: 2MImH) in ethyl acetate.

Table 2 Comparison of CL curves generated from ODI derivative-CL reactions

ODI derivative	Reaction time (s)[a]	I_{max}[b]	τ_{max} (s)	τ_{half} (s)
ODI	120.0	31.1	0.6	2.4
OD2MI	180.0	15.6	0.5	7.2
OD4MI	45.0	61.1	0.5	2.9

[a] Reaction time between TCPO and ImH derivative (ImH, 2MImH, and 4MImH) to form maximum concentrations of ODI derivatives. [b] Relative I_{max} for each experimental condition was normalized by that observed for the TCPO-CL reaction in the presence of ImH. Reaction conditions: [TCPO] = 0.1 mM, [H_2O_2] = 10.0 mM, [ImH] = 2.0 mM, [2MImH] = 2.0 mM, [4MImH] = 2.0 mM, [1-aminopyrene] = 0.3 mM, solvent: ethyl acetate.

Notes and references

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