Chemiluminescent Characters of Hydroperoxide and Dioxetanone of Coelenterate Luciferin Analog Prepared by Low-Temperature Photooxygenation

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Chemiluminescent characters of 2-hydroperoxide **2** and 1,2-dioxetanone **6** of coelenterate luciferin analog **1** prepared by low-temperature photooxygenation are described. Direct luminescence by thermal decomposition of **2** or **6** was independently observed, suggesting that the former emitted light as anionic amide **4**, on the other hand, the latter as neutral one **7**.

Chemiluminescence mechanism of coelenterate luciferin (coelenterazine; R_1 = *p*-hydroxybenzyl, R_2 = *p*-hydroxyphenyl, and R_3 = benzyl) has long been discussed.[1-4] We have been studying this mechanism and reported that direct photooxygenation of **1**[5] (R_1 = *tert*-butyl, R_2 = *p*-methoxyphenyl, and R_3 = benzyl) in CF_3CH_2OH-CH_3OH (7:3) at –78 °C afforded the hydroperoxide **2** and the dioxetanone **6**, which was shown by means of ^{13}C NMR[6] [**2**, δ 108.1 (C-2), 178.7 (C-3), and 109.8 (C-5); **6**, δ 108.7 (C-2), 169.5 (C-3), and 139.2 (C-5)] (Figure 1). These two luminescent peroxides decompose to give **8** and light at two different temperatures (**A** at –40 °C and **B** at 0 °C in Figure 2a) while the cold-photoirradiated mixture is allowed to come to room temperature. Taking thermostability into account, we temporarily assigned luminescence **A** and **B** to originate from **6** and **2**, respectively.[6] In this communication, we describe the chemiluminescent characters and conclusive assignment of these two peroxides **2** and **6** by means of luminescence spectra.

Analog **1** emitted light (475 nm) as the anionic amide **4** at 3×10^{-5} M (1 M = 1 mol dm^{-3}) when dissolved in diglyme (DGM) or DGM containing base by air oxidation. This is supported by the fluorescence spectrum of the amide **8** in basic DGM. Interestingly, even in DGM containing acetate buffer, **1** emitted light as the anionic amide **4**. After luminescence, fluorescence of 400 nm was observed from every spent solution. This means that the amide **8** is protonated even in basic DGM after luminescence. These facts are supported by the fluorescence spectra observed at 400 nm of the amide **8** in acidic or neutral DGM. Relative light yield of **1** in basic, acidic or neutral DGM was 1, 0.05 or 0.001, respectively.[7]

A 2.1 mM solution of **1** in CF_3CH_2OH-CH_3OH (7:3) was photooxygenated at –78 °C for 10 min,[8] then transferred into DGM at –78 °C, being diluted to 3×10^{-5} M.[9] We can change the luminescence conditions by this dilution. Each diluted solution was allowed to come to room temperature in about 20 min to show luminescence as indicated in Figure 2b-d. Figure 2a shows that of original solution in alcoholic solvent. After dilution with DGM, relatively smaller amount of **A** remained than **B**.[10]

The neutral DGM solution at –78 °C was allowed to come to room temperature with measuring luminescence spectra at every 10 °C between –50 °C and 10 °C (Figure 3a). It is apparent that the peak appeared at 400 nm between –50 °C and –30 °C (corresponding to luminescence **A** in Figure 2b), and at 475 nm between –10 °C and 10 °C (corresponding to luminescence **B** in Figure 2b). At –20 °C, both of these peaks are observed. The maximum intensity was recorded at 10 °C.

In the case of basic DGM solution, both of luminescence **A** (400 nm) and **B** (475 nm) are observed (Figure 2c). The maximum intensity of luminescence (475 nm) was observed at –20 °C (Figure 3b). The luminescence **B** started at –50 °C and ceased at –10 °C, overlapping the luminescence **A** at around –30 °C (Figure 2c). The intensity of 400 nm-luminescence weakly appeared relative to 475 nm-luminescence as compared with the case of neutral DGM. This may be due to the increment of intensity and the low-temperature shift of luminescence **B** under basic condition (3-fold intensity of that in neutral DGM as shown in Figure 2c).[10] Thus, the luminescence **A** of 400 nm would appear as a shoulder peak between –40 °C and –20 °C. Interestingly, 475 nm-luminescence restarted at 10 °C and this is

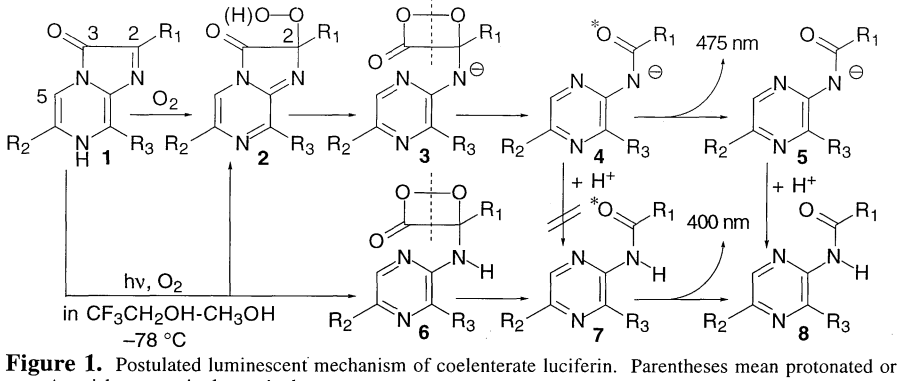

Figure 1. Postulated luminescent mechanism of coelenterate luciferin. Parentheses mean protonated or not. Asterisks mean singlet excited state.

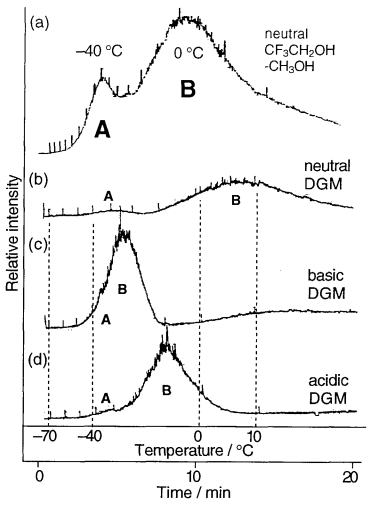

Figure 2. Chemiluminescent patterns of cold mixture on being allowed to come to room temperature. (a) without dilution [2.1 mM in CF_3CH_2OH-CH_3OH (7:3)]. (b) Neutral DGM: no additives. (c) Basic DGM: containing 0.5 vol% of 1 M *tert*-BuOK/*tert*-BuOH. (d) Acidic DGM: containing 0.5 vol% of 0.2 M acetate buffer of pH 5.6.

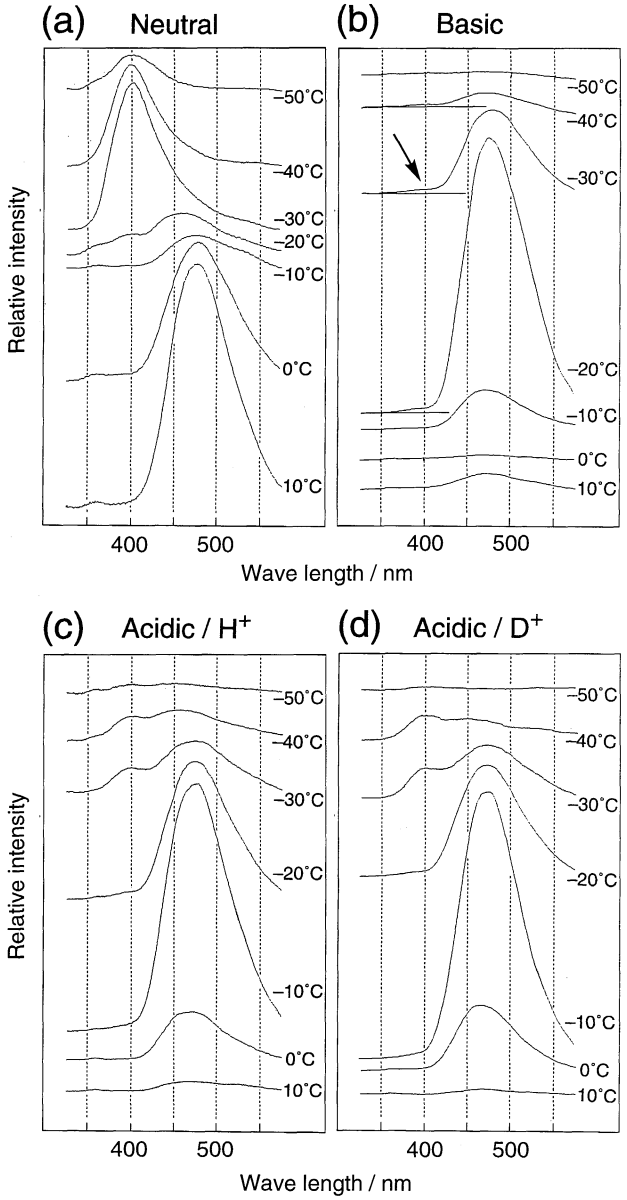

Figure 3. Chemiluminescence spectra of cold photooxygenated mixture diluted with DGM on being allowed to come to room temperature. (a), (b) and (c); see Figure 2 caption. (d) Acidic: containing 0.5 vol% of 0.2 M acetate buffer of pD 5.6. In each column, the spectra are arranged so that the highest intensity becomes identical.

attributed to luminescence of the remaining **1**.[6] The luminescence **A** of 400 nm was observed even under the basic condition.

The acidic DGM solution emitted light of 400 nm between –40 °C and –30 °C and of 475 nm between –30 °C and 10 °C (Figure 3c). The maximum intensity was observed at –10 °C.

According to the results obtained, it is obvious that the luminescence **A** of 400 nm appeared at the temperature lower than the luminescence **B** of 475 nm under all conditions; the temperature of luminescence **A** did not seem to be affected by the additives of DGM, on the other hand, that of luminescence **B** shifted below in the order of neutral, acidic and basic DGM. In the chemiluminescence at room temperature, **1** gives **4** and light (475 nm) even in acidic DGM. It implies that the protonated **2** shows luminescence of the anionic amide **4** (475 nm) even in acidic DGM. We, however, clearly observed the 400 nm-luminescence of **7** corresponding to the fluorescence of the protonated amide **8** at around –30 °C in Figure 3. We believe that **7**, the emitter of luminescence **A** (400 nm), forms directly by the decomposition of **6**, not by the protonation of **4**. If **7** formed by protonation of **4**, the process **4** to **7** should be slower in D-buffered DGM; the luminescence of **7** (400 nm) should decrease.[11] As shown in Figure 3c and 3d, the luminescence spectra of the cold acidic (pD 5.6) DGM mixture (acidified by acetate buffer prepared from CH_3CO_2D and CH_3CO_2Na in D_2O) gave almost the same spectra pattern of the acidic (pH 5.6) DGM solution.[12]

The thermally unstable dioxetanone **6** having a proton on *N*-atom directly shows luminescence **A** of 400 nm *via* **7** at lower temperature by thermal decomposition, and the hydroperoxide **2**, regardless of protonated or deprotonated form, emits light of 475 nm (luminescence **B**) *via* anion amide **4**.[13] This interpretation also satisfies the fact that the luminescence of **7** originating from **6** was observed at 400 nm even in the DGM containing *tert*-BuOK at around –30 °C though the remaining amount was small.[10]

We have concluded the assignment of the two photoproducts, hydroperoxide **2** and dioxetanone **6**, from luminescence spectra in addition to ^{13}C NMR in the previous paper.[6, 14]

References and Notes

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8 Maximum amount of the peroxides was accumulated, see Ref. 5. We observed both of luminescence **A** (400 nm) and **B** (475 nm) on the 20-min photooxygenated sample in which **1** disappeared.
9 Without dilution, sensitized-luminescence was observed which originated from remaining **1** and **8**.
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