Environmental Dependence of Chemiluminescence Using Solvatochromic Molecules

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Functional dyes are one of the most attractive materials because of their potential use in microenvironment probes, bioapplications, and optoelectronic devices. The modification of chemical structures of the functional dyes due to external stimuli such as heat and photo-irradiation affects the absorption and emission behavior of the dyes. Among them, solvatochromic molecules are attracting great interest because they are used as molecular sensors and indicators. Herein, we investigated chemiluminescence using betaine 30 and dansyl acid ethyl ester (DNSE) as solvatochromic molecules. We demonstrated chemiluminescent reactions using a mixture of betaine 30 or DNSE as the functional dye and bis(2,4,6-trichlorophenyl) oxalate as a precursor to introduce a high-energy moiety under chemiluminescence conditions in several solvents. Although betaine 30 did not exhibit fluorescence except with toluene, DNSE showed different emission colors depending on the solvent polarity, which indicated that chemiluminescence depends on the environment.

Keywords: Chemiluminescence, Solvatochromism, Betaine 30, Dansyl group

1. Introduction

Functional dyes are attracting intense interest in applications such as photo-memories, optical devices, and dimming windows [1–4] because their absorbance and fluorescence can be controlled by the external stimuli such as heat and photo-irradiation. Crenshaw et al. [5] and Pucci et al. [6] reported that the emission wavelengths of solid-state distyrene derivatives were modified by changing their aggregation mechanisms following external stress. Imato et al. have reported the development of mechanochromic materials, in which a color change was observed in response to external stress, using a polymer with a spiropyran group [7]. Furthermore, we successfully showed volume phase transition using an *N*-isopropylacrylamide derivative and detected the emission of a dansyl group [8]. Solvatochromic molecules, in which the absorption and emission wavelengths change depending on the solvent polarity, are well-known functional dyes. Several studies have

proposed their application as molecular sensors and indicators for examining solution polarity [9–12]. The solvatochromic molecules may also help develop solvatochromic materials.

Chemiluminescence is observed when a chemical moiety in the excited state is stabilized to its ground state, accompanied with the emission of fluorescence [13–16]. For example, 1,2-dioxetanedione is produced when oxalate derivatives are treated with hydrogen peroxide and an inorganic salt; subsequently, it changes to carbon dioxide with energy emission. When the dyes are mixed in the reaction system, they receive the emitted energy and exhibit fluorescence. In conventional research on chemiluminescence, the emission wavelengths were controlled by modifying the chemical structures of the dyes [15,16].

The purpose of the present study is to develop a solvatochromic dye using the chemiluminescence method, in which the wavelength of emission is controlled by

environmental conditions. To this end, we used betaine 30 [17–20] and a dansyl acid derivative [21–23] as solvatochromic molecules (Figure 1). The former, a well-known solvatochromic molecule, is a relatively highly polar molecule because it has both cationic and anionic charges within its molecular structure. Solvatochromism results due to the formation of a different molecular structure depending on polarity of solvents when the solvation manner of the polar ground state is varied with respect to the less polar excited state [17]. The dansyl group is generally used as a fluorescent labeling reagent for proteins and amino acids. Because the dansyl group has a large Stokes shift, its fluorescence quantum yield and emission wavelength are expected to change with solvent polarity. However, its solubility is quite low. Therefore, we used an esterified derivative of the dansyl group, dansyl acid ethyl ester (**DNSE**) [24, 25], to improve its low solubility. In addition, chemiluminescence reactions were performed using bis(2,4,6-trichlorophenyl) oxalate as a precursor to produce an excited intermediate with sodium carbonate and hydrogen peroxide. Figure 2 shows the chemiluminescence reaction. Although we did not visually observe chemiluminescence of betaine 30 because its fluorescence intensity was relatively low except with toluene, we showed the environmental dependence of chemiluminescence of **DNSE** using relatively low-polarity solvents.

Fig. 1. Chemical structures of betaine 30 (a) and dansyl acid ethyl ester, **DNSE** (b).

2. Experimental

2.1. Apparatus

UV-vis absorption spectroscopy measurements were performed using a UV-visible spectrophotometer (V-550, JASCO Corporation UV-2600, SHIMADU Corporation), and emission and excitation spectroscopy measurements were performed using a spectrofluorometer (FP-6200 and FP8300 JASCO Corporation) with each solvent. The excitation wavelengths for the emission spectra and the detected wavelengths for the excitation spectra of betaine 30 and **DNSE** are summarized in Tables 1 and 2, respectively. ^1H NMR (400 MHz) spectra were measured on a Bruker UltraShield Plus 400

Table 1. Summary of excitation wavelengths for emission spectra and detected wavelengths for excitation spectra of betaine 30.

Solvent	Excitation wavelength of emission spectra (nm)	Detected wavelength of excitation spectra (nm)
MeOH	500	525
acetone	352	393
EtOAc	320	383
toluene	358	440

Table 2. Summary of excitation wavelengths for emission spectra and detected wavelengths for excitation spectra of **DNSE**.

Solvent	Excitation wavelength of emission spectra (nm)	Detected wavelength of excitation spectra (nm)
NAA	356	531
DMF	359	530
MeOH	350	529
DEP	358	514
EtOH	352	526
Acetone	354	522
CH_2Cl_2	358	507
THF	351	501
EtOAc	351	501
Toluene	352	482
HFB	354	475
Hexane	342	449

Fig. 2. Chemical reactions of chemiluminescence.

spectrometer. The spectra were referenced to the proton signal of tetramethylsilane (0 ppm) in CDCl_3 .

2.2. Reagents

All commercially available reagents were used as received from TCI or Wako Pure Chemical Industries, Ltd.

2.3. Syntheses of compounds

2.3.1. Preparation of betaine 30 [17]

A mixture of 4-amino-2,6-diphenylphenol (254 mg, 972 μmol), 2,4,6-triphenylpyrylium hydrogensulfate (399 mg, 982 μmol), sodium acetate (2.02 g, 24.6 mmol), and ethanol (9.9 mL) was refluxed for 3 h. To the mixture, 1 M NaOH aq. (178 mg) was added. Then, ethanol was removed under vacuum and the crude product was washed with pure water. After filtration, the product was dried under vacuum for 88 h. The crude mixture was purified by silica gel column chromatography ($\text{CH}_2\text{Cl}_2/\text{EtOH} = 9/1$) to afford betaine 30 (44% yield) as a blue solid. The chemical reaction is shown in Figure 3a.

^1H NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$): δ 8.06 (s, 2H), 7.86–7.88 (m, 2H), 7.62–7.64 (m, 3H), 7.38–7.39 (m, 10H), 7.31–7.33 (m, 4H), 7.16 (t, $J = 7.5$ Hz, 4H), 7.08 (t, $J = 7.5$ Hz, 2H), 6.45 (s, 2H).

2.3.2. Preparation of DNSE [24]

Dansyl chloride (403 mg, 1.48 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (225 mg, 1.48 mmol) were dissolved in ethanol (20 mL). The reaction mixture was stirred at room temperature. After 17 h, the mixture was added

dropwise to water. The product was extracted with CH_2Cl_2 . The organic layer was dried over Na_2SO_4 . After removing the solvent under vacuum, the mixture was subjected to silica gel column chromatography ($\text{CH}_2\text{Cl}_2/\text{hexane} = 4/1$) to obtain DNSE (50% yield) as a yellow solid. The chemical reaction is shown in Figure 3b.

^1H NMR (400 MHz, CDCl_3 , 25 $^\circ\text{C}$): δ 8.59 (d, $J = 8.0$ Hz, 1H), 8.26 (dd, $J = 7.3$ and 1.2 Hz, 1H), 8.11 (d, $J = 8.0$ Hz, 1H), 7.71 (dd, $J = 10.1$ and 9.7 Hz, 1H), 7.69 (dd, $J = 10.1$ and 9.7 Hz, 1H), 7.30 (d, $J = 8.0$ Hz, 1H), 4.05 (q, $J = 8.0$ Hz, 2H), 2.84 (s, 6H), 1.15 (t, $J = 8.0$ Hz, 3H).

2.4. Chemiluminescence reaction

A mixture of bis(2,4,6-trichlorophenyl) oxalate (50 mg, 110 μmol), sodium carbonate (100 mg, 940 μmol), dye (0.1 eq. of betaine 30 or DNSE), and solvent (10 mL) was treated with an ultrasonic cleaner. After 5 min, 35% hydrogen peroxide aq. (3 mL) was added to the mixture.

3. Results and discussion

3.1. Optical properties of betaine 30 based on solvents

We measured the UV spectra of betaine 30 (1.0×10^{-4} M) using methanol, acetone, ethyl acetate, and toluene as solvents (Figure 4). Table 3 shows a summary of maximum wavelengths of absorption (λ_{Abs}), emission (λ_{Em}), and excitation (λ_{Ex}) in each solution. The value of λ_{Abs} red-shifted from 525 nm (MeOH) to 738 nm (ethyl acetate) as the polarity of the solvent except toluene (429 nm) was increased. In addition, concentration quenching was observed in the excitation spectrum of toluene.

Fig. 3. Schemes of syntheses of betaine 30 (a) and DNSE (b).

Fig. 4. UV spectra of betaine 30 measured in methanol (solid line), acetone (broken line), ethyl acetate (dotted line), and toluene (chain line).

Figure 5 shows the emission and excitation spectra of betaine 30 using methanol, acetone, ethyl acetate, and toluene (1.0×10^{-4} M). Table 3 shows summary of λ_{Abs} , λ_{Em} , and λ_{Ex} for these spectra. For acetone and ethyl acetate, although λ_{Abs} was detected at 685 nm, 738 nm (Figure 4), the corresponding emission was not observed. Instead, emission at 467 nm was detected for excitation at 400 nm for methanol. For the other solvents, emissions corresponding to the absorption of betaine 30 were observed. For fluorescence in the visible range, the intensity of emission in toluene was the highest, although the intensity of the detected emissions was relatively weak.

Fig. 5. Emission (blue) and excitation (red) spectra of betaine 30 in methanol (solid line), acetone (broken line), ethyl acetate (dotted line), and toluene (chain line).

Table 3. Summary of maximum wavelengths of absorption (λ_{Abs}), emission (λ_{Em}), and excitation (λ_{Ex}).

Solvent	λ_{Abs} (nm)	λ_{Ex} (nm)	λ_{Em} (nm)
MeOH	525	500	522
acetone	394, 685	352	420
EtOAc	399, 738	320	370
toluene	429	358	437

3.2. Chemiluminescence using betaine 30

Although the intensity of betaine 30 was low, we performed its chemiluminescence reaction using the method described in Section 2.4. We demonstrated the reaction using methanol, acetone, ethyl acetate, and toluene solvents; however, we could visually detect chemiluminescent emission only in toluene (Figure 6). In other solvents, unfortunately, we did not observe chemiluminescent emission because of the low intensity of the emission.

Fig. 6. Photo image of the mixture of reagents for chemiluminescence using betaine 30 as a dye in toluene.

3.3. Optical properties of DNSE based on solvents

Next, we investigated the optical properties of DNSE using *N*-methylacetamide (NAA), dimethylformamide (DMF), methanol, diethyl phthalate (DEP), ethanol, acetone, CH_2Cl_2 , ethyl acetate, toluene, hexafluorobenzene (HFB), and hexane as the solvent. The solute concentration of DNSE was 1.0×10^{-4} M for each solvent [25]. Figures 7 and 8 show the UV spectra and emission and excitation spectra, respectively, obtained with each solution. The values of λ_{Abs} , λ_{Em} , and λ_{Ex} with permittivity are summarized in Table 4. The λ_{Abs} was detected between 330 and 360 nm for all solutions. However, λ_{Em} shifted to longer values with increasing polarity of the solvent. The λ_{Em} was detected at 449 nm in hexane (the lowest-polarity solvent) and at 529

Fig. 7. UV spectra of DNSE in NAA (pink solid line), DMF (pink dotted line), methanol (pink broken line), DEP (pink one-dot chain line), ethanol (pink two-dot chain line), acetone (blue solid line), CH_2Cl_2 (blue dotted line), THF (pink short broken line), ethyl acetate (blue broken line), toluene (blue one-dot chain line), HFB (blue two-dot chain line), and hexane (blue short broken line).

Fig. 8 Emission (blue) and excitation (red) spectra of **DNSE** in NAA (bold solid line), DMF (bold dotted line), methanol (bold broken line), DEP (bold one-dot chain line), ethanol (bold two-dot chain line), acetone (narrow solid line), CH_2Cl_2 (narrow dotted line), THF (bold short broken line), ethyl acetate (narrow broken line), toluene (narrow one-dot chain line), HFB (narrow two-dot chain line), and hexane (narrow short broken line).

Table 4. Summary of maximum wavelengths of absorption (λ_{Abs}), emission (λ_{Em}), excitation (λ_{Ex}), and the value of permittivity (E).

Solvent	λ_{Abs} (nm)	λ_{Em} (nm)	λ_{Ex} (nm)	E (eV)
NAA	337	529	356	2.34
DMF	334	530	359	2.34
MeOH	334	529	350	2.34
DEP	338	514	358	2.41
EtOH	336	524	352	2.37
acetone	360	520	354	2.38
CH_2Cl_2	347	506	358	2.45
THF	336	504	351	2.46
EtOAc	335	501	351	2.48
toluene	337	483	352	2.57
HFB	338	474	354	2.62
hexane	334	449	342	2.76

nm in NAA (the highest-polarity solvent). Moreover, large Stokes shifts were detected with a maximum of 173 nm for NAA solution and a minimum of 107 nm for hexane solution (Figure 8). Furthermore, the intensity of fluorescence in the lower-polarity solvents was weaker than that in higher-polarity solvents.

To summarize the solvent effect on the emission of **DNSE**, we plotted the fluorescent energies using the relative dielectric constants [26] for each solvent. The fluorescent energies (E) were calculated using the following formula:

$$E = hc / \lambda,$$

where h and c are the Planck constant and the speed of light, respectively. Figure 9 shows the plot of the calculated fluorescent energies with respect to the relative dielectric constants. The fluorescent energies were relatively higher in lower-polarity solvents. In contrast, the

Fig. 9. Plots of the fluorescence energies of **DNSE** in NAA (blue circle), DMF (yellow circle), MeOH (red circle), EtOH (blue square), acetone (yellow square), CH_2Cl_2 (red square), THF (blue triangle), EtOAc (yellow triangle), toluene (red triangle), HFB (blue rhombus), and hexane (yellow rhombus) solution with respect to the relative dielectric constants.

fluorescence energies of **DNSE** in solvents with relatively higher relative dielectric constants were observed at a constant value of ~ 2.3 eV.

3.4. Chemiluminescence using ethyl dansylate

We performed chemiluminescent experiments using **DNSE** in NAA, DMF, methanol, DEP, ethanol, acetone, CH_2Cl_2 , THF, ethyl acetate, toluene, HFB and hexane. Figure 10 shows the photos of the reaction mixtures in each solvent taken after adding hydrogen peroxide *aq*. No light emission was detected visually in the NAA, DMF, methanol, ethanol, and acetone EtOAc, and hexane solutions, while the emission was detected visually in DEP, CH_2Cl_2 , THF, toluene and HFB. The emission colors of the solutions owing to chemiluminescence are relatively well consistent with the fluorescent wavelengths detected in the solution phase (Table 4), which indicates that **DNSE** was excited by 1,2-dioxetanedione produced from bis(2,4,6-trichlorophenyl) oxalate in the organic phase. Surprisingly, the lifetime of the THF emission instantaneously decreases when hydrogen peroxide was added. Although we

Fig. 10. Photo images of the solutions after chemiluminescent reaction using **DNSE** in THF, DEP, CH_2Cl_2 , toluene and HFB solution.

could not completely understand this phenomenon, we hypothesize that energy transfer from 1,2-dioxetanedione to **DNSE** occurred momentarily in the THF and water solution because of their miscibility. Note that, in highly polar solvents, the emission of **DNSE** was not visualized, probably because the rate of chemiluminescent reaction is too fast to visually detect the emission caused by the miscibility of their solvents with water in comparison with the case observed using low-polarity solvents. Moreover, no emission was observed with ethyl acetate, although it is immiscible with water. This is probably because the reaction of ethyl acetate with hydrogen peroxide was faster than the oxidation of bis(2,4,6-trichlorophenyl) oxalate. As a result, **DNSE** was not excited because 1,2-dioxetanedione was not produced.

4. Conclusion

We used betaine 30 and **DNSE** as solvatochromic molecules to investigate the dependence of chemiluminescence on solvent polarity. Although absorptions of betaine 30 were detected within 340–900 nm depending on the solvent polarity, the intensity of fluorescence was relatively low and chemiluminescence was not observed visually except for toluene. **DNSE** showed chemiluminescence with the solvents that are immiscible with water, producing several colors depending on the solvent polarity. It is possible that the lifetime of chemiluminescence in the solvents miscible with water is quite short and is not sufficient to visualize because the emission of **DNSE** in THF solution was observed only for a moment. Although it is necessary to optimize chemiluminescence conditions using solvatochromic molecules, we showed that it is possible to modify the chemiluminescence of dyes by varying the microenvironment.

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