Fluorescence and Chemiluminescence Behavior of Distyrylbenzene Bearing Two Arms of Dipicolylaminomethyl Groups: Interactions with Zinc Ion and ATP

Jiro Motoyoshiya,*[1] Jun-ya Wada,[1] Keiko Itoh,[1] Kazuaki Wakabayashi,[1] Takayuki Maruyama,[1] Kazuki Ono,[1] Kota Fukasawa,[1] Tetsuya Fujimoto,[1] Yuji Akaiwa,[2] Eiji Nonaka[2]

[1]*Applied Molecular Chemistry Course, Department of Chemistry and Materials,*

Faculty of Textile Science and Technology, Shinshu University, 3-15-1, Tokida, Ueda, Nagano 386-8567, Japan

[2]*ALCARE Co. Ltd. Medical Engineering Laboratory, 1-21-10 Kyojima, Sumida-ku, Tokyo 131-0046, Japan*

E-mail: jmotoyo@shinshu-u.ac.jp

ABSTRACT

The absorption and fluorescence spectral study of the distyrylbenzene bearing two arms of the dipicolylaminomethyl groups, the effective ligands for Zn^{2+}, was studied in the presence of Zn^{2+} and ATP. Upon complexation of the distyrylbenzene with zinc ions in acetonitrile, enhancement of the fluorescence intensity was observed due to inhibition of intramolecular PET (photo-induced electron transfer) quenching, but no effect was found in aqueous media because the equilibrium laid to the free form of the ligands. In contrast, the addition of ATP disodium salt was effective to enhance the fluorescence intensity of the combination of the distyrylbenzne and Zn^{2+} in aqueous media. This assembly was applied to the peroxyoxalate chemiluminescence system and a significant increase in the intensity was observed, which provides a potential detection for ATP by chemiluminescence.

Keywords: Fluorescence; Chemiluminsecence; Distyrylbenzene; Dipicolylaminomethyl group; zinc ion; Complex; ATP

1. Introduction

Due to the extremely increasing importance of the detection and visualization of biological substrates that are playing a critical role in living cells, a number of fluorescent probes with sensitive sites to such molecules have been developed [1,2]. Of the substrates taking part in various biological processes, much attention has focused on the development of fluorescent probes for

phosphorylated molecules [3]. Since adenosine triphosphate (ATP) is one of the most important molecules, many chemosensors for ATP have been documented [4]. Most of these probes make their fluorescence intensities stronger by the chemical interaction with ATP and the sensitive site in the functionalized fluorescent molecules, a representative example is a zinc complex of 9,10-bis(dipicolylaminomethyl)anthracene [5-7]. The mechanism of the fluorescence enhancement of this ATP chemosensor involves control of the fluorescence quenching caused by an intramolecular PET (photo-induced electron transfer) between an electron-donating ligand and the fluorescent core [8.9]. The stabilization of the complex reduces the electron-donating ability of the nitrogen-containing ligand, such as a dipicolylamino group, to inhibit the intramolecular fluorescence quenching. On the other hand, the strongly fluorescent distyrylbenzene (DSB) is also expected to be a candidate for the framework of a fluorescent probe because the fluorescence quantum yield for the parent DSB is known to be 1.00 higher than that for anthracene (0.33) [10-12]. In this paper, we report the absorption and fluorescence spectral behavior in the interaction of the distyrylbenzene bearing two arms of the dipicolylaminomethyl groups with Zn^{2+} and ATP, and the application of this combination to the peroxyoxalate chemiluminescence (PO-CL) system was also conducted.

2. Results and Discussion

2.1 Preparation and ^1H NMR Spectra in the Presence of Zn^{2+}

The central compound, 1,4-bis(dipicolylaminomethyl)-2,5-distyrylbenzene (**1**), was prepared by the reaction of dipicolylamine and 1,4-dibromomethyl-2,5-distyrylbenzene, the latter was obtained by bromination of known 1,4-dimethyl-2,5-distyrylbenzene prepared by the Horner-Wadsworth-Emmons reaction of 1,4-bis(diethylphosphonomethyl)-2,5-dimethylbenzene and benzaldehyde [11]. Upon treatment of thus prepared **1** with zinc nitrate in a mixed solution of methanol, dichloromethane and acetone, the complex (**1-Zn**) was formed (Fig. 1). The capture of zinc ions by the dipicolylaminomethyl groups of **1** was confirmed by the ^1H-NMR spectra as shown in Fig. 2.
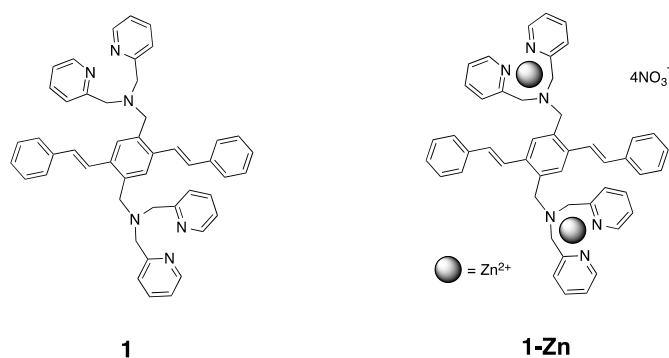
Fig. 1. Chemical structure of **1** and its zinc complex (**1-Zn**).

In comparison of some signals in the dipicolylamino groups of **1** with those of **1-Zn**, the methylene protons lying between the nitrogen atoms and the pyridine rings and 5'-H pyridine protons are not only shifted to the lower fields due to chelation of the positively charged zinc ions, but also changes in their shapes with broadening and splitting were observed as previously reported in the zinc complex of the acridine derivative with two pendant arms of dipicolylaminomethyl groups [14].
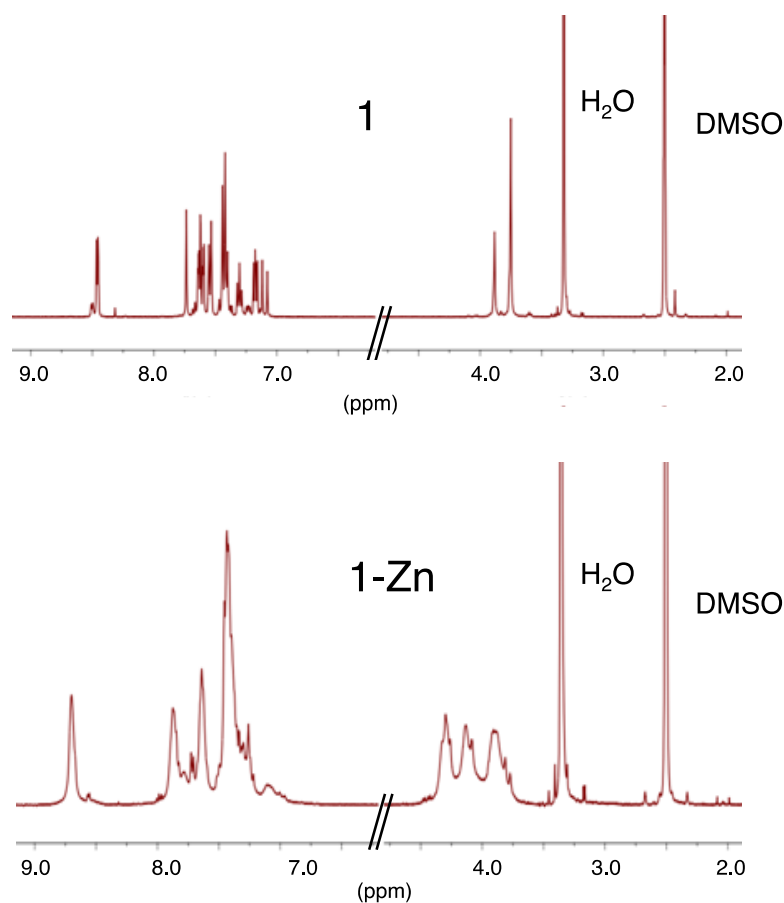

Fig. 2. ^{1}H NMR spectral change of coordination of **1** upon complexiation with Zn^{2+} in DMSO-d$_6$.

2.2 Absorption and Fluorescence Spectral Study of 1 in the Presence of Zinc Ions and ATP-disodium salt (ATP-Na₂)

Compound **1** and Zn complex, **1-Zn**, showed similar absorption spectra with peaks around 260 nm and 350 nm in acetonitrile and in 1:1 THF/HEPES buffer as shown in Fig. 3a and 3b. A remarkable difference in the fluorescence spectra depending on the media was found as shown in Fig. 3c and 3d. The emission spectra of **1** and **1-Zn** in acetonitrile afforded two emission maxima at 400 nm and a larger peak at 423 nm. The fluorescence quantum yield of **1** was estimated as 0.16 in acetonitrile, while that of **1-Zn** was estimated to be 0.70, therefore, increasing ca. 5 times by complexation of Zn^{2+} as shown in Fig. 3c. This is probably due to suppression of the PET fluorescence quenching occurred in **1-Zn**. On the other hand, the fluorescence intensity of **1** increased ca. 3.6 times in THF/buffer solution compared to that in acetonitrile as shown in Fig. 3d. In addition, it is important to note that the difference in fluorescence intensity between **1** and **1-Zn** was not observed in this aqueous media (Fig. 3d). This difference in spectral behavior means that while the complexation of zinc ions to the dipicolylamino groups is preferential in acetonitrile, the equilibrium lies to the free form of the ligand in the aqueous media.
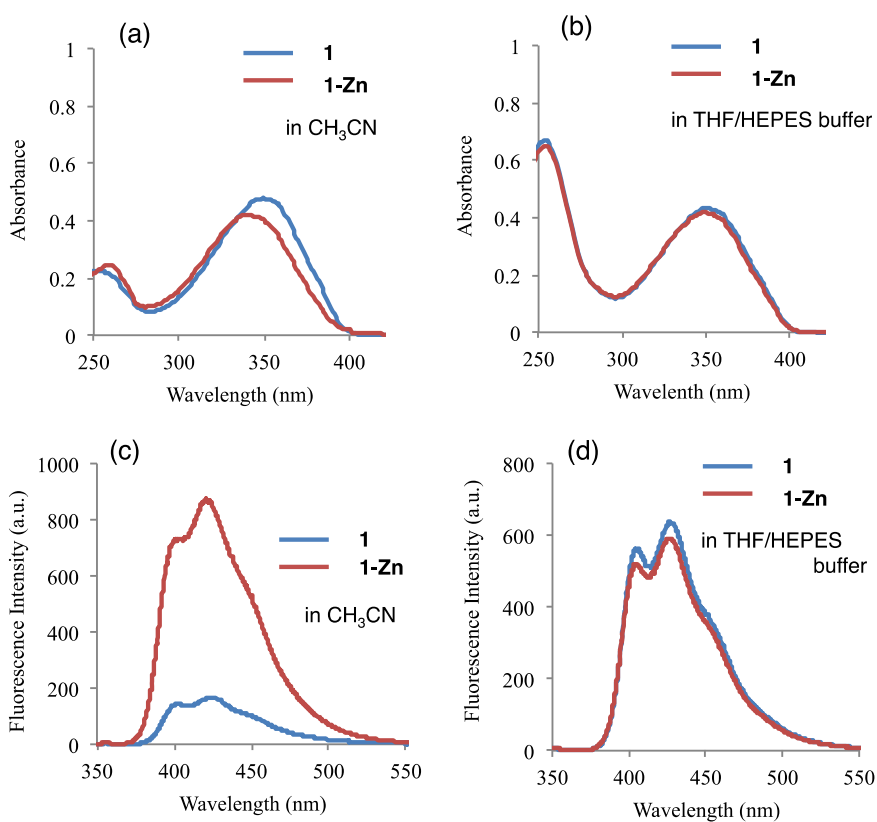

Fig. 3. Absorption and fluorescence spectra of **1** and **1-Zn**. [**1**] = [**1-Zn**] =1x10⁻⁵ M. (a)

Absorption spectra of **1** in CH$_3$CN. (b) Absorption spectra of **1-Zn** in 1:1 THF/HEPES buffer solution. (c) Fluorescence spectra of **1** excited at 355 nm in CH$_3$CN. (d) Fluorescence spectra of **1-Zn** excited at 350 nm in 1:1 THF/HEPES buffer solution.

Based on this spectral study, the interaction of **1-Zn** and disodium adenosine triphosphate (ATP-Na$_2$) in the aqueous media was investigated. There was almost no change in the absorption spectra for the DSB framework at 350 nm when various amounts of ATP-Na$_2$ were added to the solution of **1-Zn** (Fig. 4a), whereas the absorption of ATP at 260 nm increased with its increasing concentration. In the fluorescence spectra, the gradual enhancement of the intensity at 423 nm arising from the DSB framework was observed except for one decrease for 0.5 eq. of ATP-Na$_2$ as shown in Fig. 4b. This fluorescence enhancement was totally due to the DSB core but not due to ATP-Na$_2$, considering the fluorescence of ATP-Na$_2$ was much weaker than that of the DSB framework. Upon the addition of 8 eq. of ATP-Na$_2$ to the complex, the intensity became greater by more than two times compared to that in the absence of ATP-Na$_2$. In contrast, in the absence of Zn^{2+} almost no change in the fluorescence intensity of **1** was observed upon addition of ATP-Na$_2$ as shown in Fig. 4c. Therefore, the fluorescence enhancement in the ensemble **1**-Zn^{2+}-ATP-Na$_2$ is explained by canceling of the intramolecular fluorescence quenching due to stabilization of the complex by the additional combination with the phosphate moiety of ATP [15].
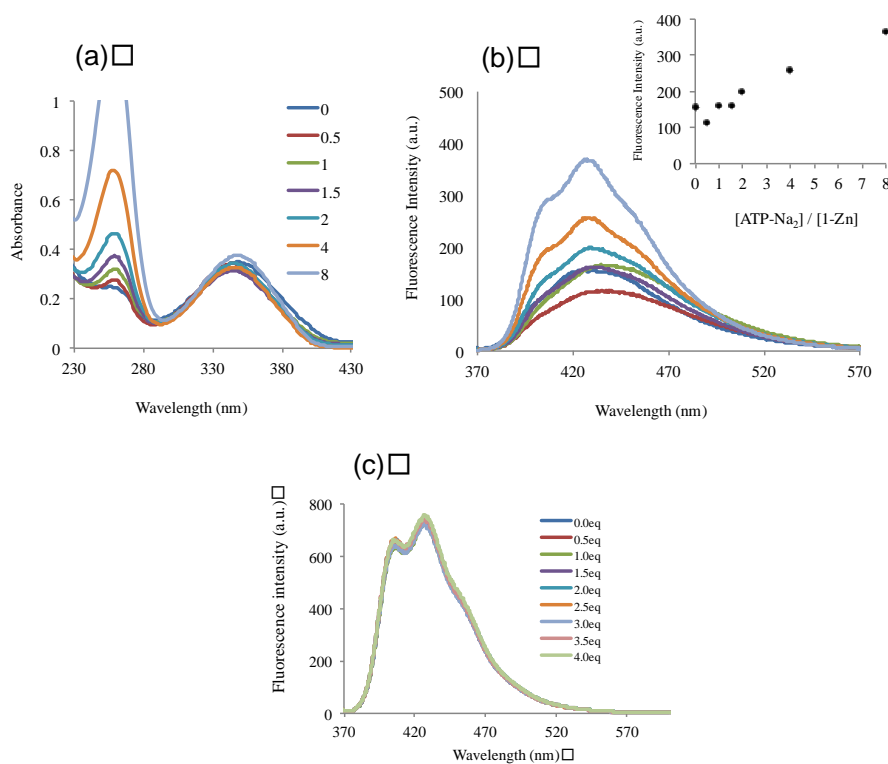

Fig. 4. Absorption and fluorescence spectra of **1-Zn** observed upon addition of ATP-Na$_2$.

[**1-Zn**]=1.0 x10^{-5} M and [ATP-Na$_2$] = 0, 0.5 x 10^{-5} M, 1.0 x 10^{-5} M, 1.5 x 10^{-5} M, 2.0 x 10^{-5} M, 4.0 x10^{-5} M, 8.0 x 10^{-5} M in H$_2$O. (a) Absorption spectra. (b) Fluorescence spectra excited at 350 nm. Inset: Plot of the fluorescence intensity at 429 nm vs. the ratios of [ATP-Na$_2$] / [**1-Zn**]. (c) Fluorescence spectra of **1** observed upon addition of ATP-Na$_2$ in the absence of Zn^{2+}.

2.3 Imaging of the Interaction of *1-Zn* and Bacteria

As has been known that some fluorescent probes bearing dipicolylamino groups adhere to bacteria to visualize their distribution [16,17], we made an attempt to apply this complex to accentuate one type of bacteria, *Staphylococcus aureus*, which exists in many human bodies and causes various diseases [18]. We found that the complex **1-Zn** combined with this bacterium and the area where the bacterium is distributed on the slide glass emitted a fluorescence as shown in Fig. 5a-1. A distinct visualization by the DSB fluorescence was successful when compared with the blank (Fig. 5a-3) without using **1-Zn**. For comparison, we treated the known zinc complex of 9,10-bis(dipicolylaminomethyl)anthracene in a similar manner (Fig. 5b-1). Although both probes demonstrated the bacteria distribution by means of their fluorescence, drawing a parallel between their fluorescence spectra, Fig. 5a-2 and Fig. 5b-2, revealed the predominance of **1-Zn** over the anthracene probe regarding the fluorescence intensity, the former of which was found to be stronger by approximately two times than that of the latter. However, the shortcoming of **1-Zn** is the lack of a photoresistance because the fluorescence of **1-Zn** gradually faded due to UV irradiation after several minutes [19].
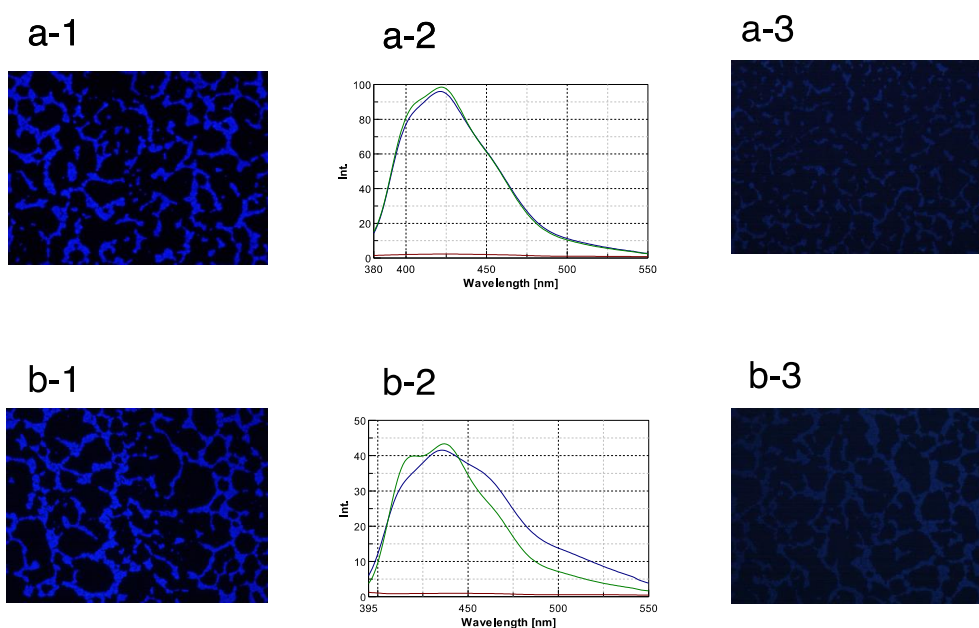
Fig. 5. Pictures of fluorescence microscope and fluorescence spectra of bacterium, *Staphylococcus aureus*, connected with two fluorescence probes. (a-1) Picture taken upon interaction between **1-Zn** and bacteria. (a-2) Fluorescence spectra showing the **1-Zn**-bacteria interaction. The red line lying at the bottom is the blank without using **1-Zn**. The blue line indicates the spectrum after dipping the sample on the slide glass in MeOH for 2h and then washing with water. The green line is the spectrum after dipping the sample on the slide glass in MeOH for 4h and then washing with water. (a-3) Picture of the bacteria without **1-Zn** (blank). (b-1) Picture of the zinc complex showing 9,10-bis(dipicolylaminomethyl)anthracene-bacteria interaction. (b-2) Fluorescence spectra showing the zinc complex of 9,10-(dipicolylaminomethyl)anthracene-bacteria interaction. The red, blue, and green lines indicate the spectra after treatment similar to a-2. (c-3) Picture of the bacteria without the zinc complex (blank).

2.4 Application to the Peroxyoxalate Chemiluminescence System

Many fluorescent compounds can be used as the fluorophores for the peroxyoxalate chemiluminescence (PO-CL) which takes place by the interaction of the high-energy intermediate, generated by the reaction of an active oxalate and hydrogen peroxide, and the fluorescent compound as an energy acceptor [20-25]. Therefore, the fluorescent pair of **1-Zn** and ATP-Na$_2$ is also expected to be applicable for this chemiluminescence system.

When a solution of alkaline hydrogen peroxide was added to a mixture of bis(*p*-chlorophenyl) oxalate, **1-Zn**, and ATP-Na$_2$ in a 1:1 THF/HEPES buffer solution, the chemiluminescence reaction occurred, and the photons emitted from the reaction were measured by a photomultiplier tube connected to a photon counting system. Fig. 6a shows the chemiluminescence profiles recorded by changing the ATP-Na$_2$ concentration, the curves plotted with the arbitrary chemiluminescence intensity as the ordinate and time as the abscissa. The chemiluminescence reaction proceeded very fast, and the maximum intensity appeared within 1 second and the light emission completely terminated after 5 seconds. In spite of a very weak chemiluminescence, the chemiluminescence quantum yield calculated from the integration of the curves in Fig. 6a increased with the increasing ATP-Na$_2$ concentration, and the maximum was detected at [ATP-Na$_2$]/[**1-Zn**] = 2/1 as shown in Fig. 6b, but the intensity decreased upon the addition of 4 eq. of ATP-Na$_2$. Appearance of the maximum of the intensity at 2 eq. of ATP-Na$_2$ is different from the case of the fluorescence measurement (Fig. 6b), which is probably because the complex of **1-Zn**-ATP-Na$_2$ consists of a 2:1 assembly and only interacted with the high-energy intermediate to emit light. On the other hand, when the free ligand **1** and ATP-Na$_2$ were applied to the PO-CL, the chemiluminescence intensity gradually decreased by the addition of ATP-Na$_2$ (Fig. 6c and Fig. 6d), which shows that the chemiluminescence

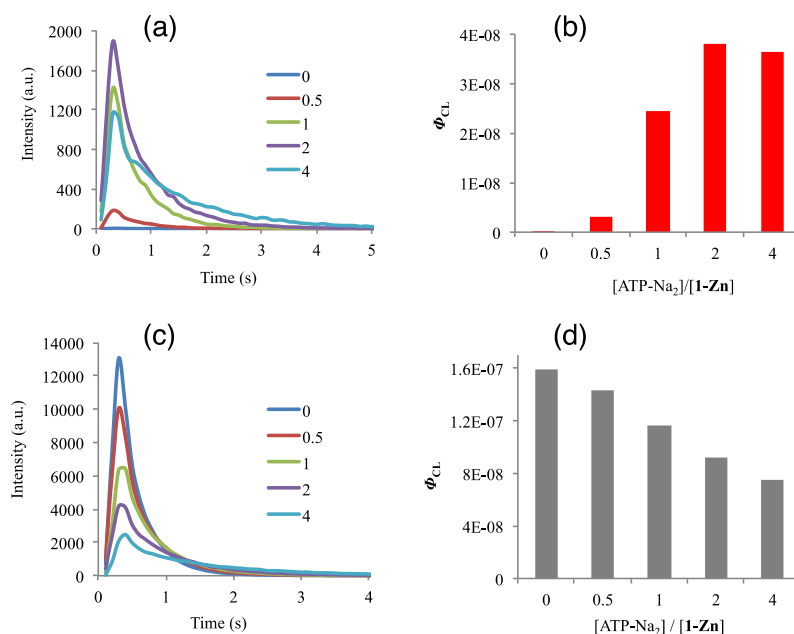enhancement is a result of the interactions with ATP-Na$_2$ and **1-Zn**.

Fig. 6. Application of the **1-Zn**-ATP interaction to the peroxyoxalate chemiluminescence system. Conditions: [bis(4-chlorophenyl) oxalate] = 5.0 x 10^{-3}M, [H$_2$O$_2$] = 0.1 M, [Na$_2$CO$_3$] = 2.5 x 10^{-3} M, [**1-Zn**] = 1.25 x 10^{-3} M, [ATP-Na$_2$] = 0.5 x 10^{-3} M, 1.0 x 10^{-3} M, 2.0 x 10^{-3} M, and 4.0 x 10^{-3} M in the THF/HEPES (pH = 8.1)　(1/1) solutions.　(a) Chemiluminescence profiles of the interactions of **1-Zn** and ATP. (b) Enhancement of the chemiluminescence quantum yields calculated by the areas of the curves depicted in (a). (c) Chemiluminescence profiles for the effect of ATP-Na$_2$ without Zn^{2+}.

The plausible mechanism of this chemiluminescence enhancement is described in Scheme 1. The complex **1-Zn** incorporated with ATP is strongly fluorescent due to canceling of the PET fluorescence quenching, which acted as a fluorophore in the PO-CL system. In the absence of ATP, **1** and **1-Zn** are in an equilibrium state in aqueous media with a very weak fluorescence due to the intramolecular PET fluorescence quenching, resulting in an ineffective chemiluminescence. It is important to note that the chemiluminescence quantum yield maximally increased by 65 times compared to that observed in the absence of ATP-Na$_2$, and such an enhancement is much greater than that observed in the fluorescence spectra. This difference is based on the different excitation paths: namely, the fluorescence emission is caused by irradiation, while the excited molecules are generated from the chemical reactions during the chemiluminescence. The mechanism of the typical PO-CL is evidenced by many investigations that involve an electron transfer called a CIEEL (chemically initiated electron exchange luminescence) process [26]. The specific characteristic of

the chemiluminescence has a high dependence on the reaction fields where an electron transfer is taking place. The complex formation of **1-Zn** and ATP in the reaction mixture might contribute to make the CL reaction favorable for the interaction with the high-energy intermediate and **1-Zn**-ATP as well as an electron transfer, which promote the chemi-excitation of the DSB framework. At present the chemiluminescence method is less sensitive than the fluorescence method because of difficulty of adjustment of the conditions suitable for the chemiluminescence reaction, but improvement will be possible by further investigation focusing on an analytical point of view.
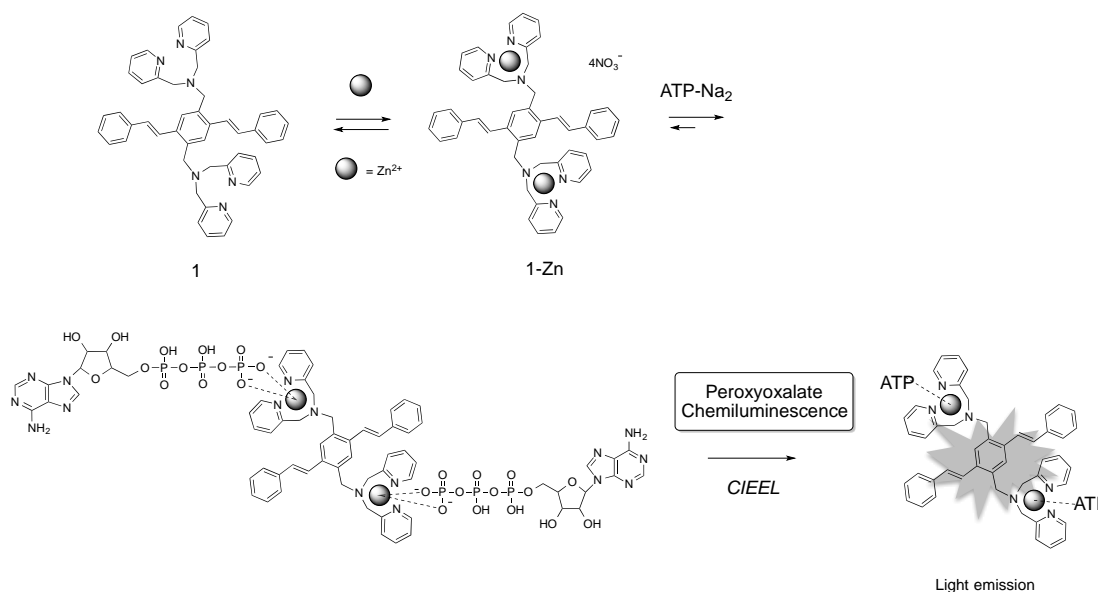
Scheme 1. A plausible mechanism of chemiluminescence enhancement by the interaction of **1-Zn** and ATP-Na$_2$.

3. Conclusion

In summary, we prepared distyrylbenzene (**1**) with two arms of dipicolylaminomethyl groups at the central benzene ring and its zinc complex (**1-Zn**), and their fluorescence and chemiluminescence behavior with the interaction of ATP was studied. The combination of **1-Zn** and ATP-Na$_2$ enhanced the fluorescence intensity. Furthermore, the application of this combination to the PO-CL system provided a contrast in the CL behavior between in the absence and presence of ATP-Na$_2$. Consequently, this study presents an example that the fluorescence probes designed for various target species are potentially applicable to the chemiluminescence system which is superior to the fluorescence measurements in certain circumstances.

4. Experimental

4.1 Materials and Equipment

Commercially available reagents were used without further purification. Solvents were distilled before use. Melting point were determined using a hot stage microscope apparatus and were uncorrected. Proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra were measured on a Bruker AVANCE-400 at 400 MHz for ^{1}H-NMR, and 100 MHz for ^{13}C-NMR, respectively, in $CDCl_3$. The chemical shifts (δ) of the ^{1}H and ^{13}C NMR spectra are reported in ppm downfield from TMS as an internal standard or from the residual solvent peak. Coupling constants (*J*) are reported in Hz. Absorption and fluorescence spectra were recorded on a U-3310 spectrometer (Hitachi) and on a RF-5000 spectrometer (Shimadzu), respectively. Mass spectra were taken with a Bruker Daltonics micro TOF II attached with APCI-Direct Probe and ESI ion sources. Chemiluminescence quantum yields (Φ_{CL}) were measured by a photon-counting method using a Hamamatsu Photonics R464 photomultiplier connected to a photon-counting unit (C3866) and a photon-counting board M8784 according to a previously reported procedure [25]. Luminol chemiluminescence was used as a standard in DMSO for the calibration of the photomultiplier tube [27].

4.2 Preparation of 1,4-di(dipicolylaminomethyl)-2,5-distyrylbenzene (1)

A suspension of 1,4-dimethyl-2,5-distyrylbenzene (3.00 g, 9.66 mmol), *N*-bromosuccinimide (NBS) (3.79 g, 21.29 mmol), and a catalytic amount of benzoyl peroxide in benzene (90 ml) was heated under reflux for 18 h. After cooling, removal of the insoluble residue by filtration and of the solvent by evaporation gave the crude product, which was washed by methanol to give 1,4-di(bromomethyl)-2,5- distyrylbenzene as yellow crystals (1.75 g, 39 %). ^{1}H NMR ($CDCl_3$) δ 4.65 (s, 4H), 7.16 (d, 2H, J = 16.1 Hz), 7.38 (d, 2H, J = 16.1 Hz), 7.42 (t, 6H, J = 8.0 Hz), 7.52 (d, 4H, J = 8.0 Hz), 7.64 (s, 2H).

A solution of thus prepared 1,4-di(bromomethyl)-2,5-distyrylbenzene (1.75 g, 3.74 mmol) and dipicolyamine, (1.65 g, 8.27 mmol), prepared by the reaction of 2-pyridine carboxaldehyde and 2-picolylamine in the presence of sodium borohydride in methanol, and triethylamine (1.90 g, 18.80 mmol) was heated at 70°C for 24 h under a nitrogen atmosphere. After cooling and removal of the solvent under a reduced pressure, the residue was washed with ether and ethanol to remove the unreacted reagents and the by-product, and the white powder of **1** was obtained (1.48 g, 56 %): this amorphous powder did not show a distinct melting pint, ^{1}H NMR ($CDCl_3$) δ 3.82 (s, 8H), 3.87 (s, 4H), 6.99 (d, 2H, J = 16.1 Hz), 7.06 (m, 4H), 7.29 (m, 4H), 7.39 (m, 4H), 7.45-7.56 (m, 16H), 7.64 (s, 2H), 8.47-8.52 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 61.09, 122.31, 123.55, 127.18, 129.03,

136.74,149.32, 160.02. □□□□ (ESI) m/z calcd for $C_{48}H_{44}Na$: 727.3520 [M+Na]$^+$; found 727.3520.

*4.3 Preparation of zinc complex of 1,4-di(dipicolylaminomethyl)-2,5-distyrylbenzene (**1-Zn**)*

A solution of **1** (301 mg, 0.426 mmol) and zinc nitrate hexahydrate (0.271 g, 0.895 mmol) in a mixed solvent (24 ml, methanol: dichloromethane: acetone = 1:1:1) was stirred at room temperature for 2.5 h. After removal of the solvent, the residue was washed with methanol to remove unreacted reagents to give the complex of **1-Zn** (0.41 g, 88 %). ^{1}H NMR (DMSO-d_6) δ 3.75-4.40 (m, 12H), 7.35-7.88 (m, 28H), 8.68-8.70 (m, 4H).

4.4 Determination of fluorescence quantum yield

9,10-Diphenylanthracene (DPA) was used as a standard [$\Phi_F = 0.82$ in benzene: Lit. J. V. Morris, M. A. Mahaney, J. R. Huber, J. Phys. Chem. 80 (1976) 969, Fluorescence Quantum Yield Determinations. 9,10-Diphenylanthracene as a Reference Standard in Different Solvents] by excitation at 375 nm. Compound **1** and DPA were dissolved in acetonitrile and benzene, respectively, at 10^{-6} M and measured their absorptions at 375 nm. Thus obtained absorptions (Abs) and the integrated areas (S) of the emission spectra obtained by excitation at 375 nm were used for estimation of the fluorescence quantum yields according to the following equation involving the values of refractions (*n*) for the corresponding solvents (acetonitrile: 1.34, benzene: 1.50).

$$\Phi_{F\ Compound\ 1} / \Phi_{F\ DPA} = (S_{Compound\ 1} \times Abs_{DPA} / S_{DPA} \times Abs_{Compound\ 1}) \times (n_{acetonitrile})^2 / (n_{benzene})^2$$

4.5 Measurement of chemiluminescence quantum yields

Bis(4-chlolophenyl) oxalate was prepared from the reaction of oxalyl chloride and two equivalent of 4-chlorophenol in the presence of triethylamine in benzene. This oxalate was recrystallized from benzene. The photons, generated from the reactions by mixing the three solutions A, B, and C (vide infra) in a quartz cell placed in front of the photomultiplier at 25°C, were counted with the photon-counting system. The average of the values obtained by more than three times measurements was used for the calculation of Φ_{CL}.

The three solutions were prepared as follows:

A: a solution of bis(4-chlolophenyl) oxalate (1.5 x 10^{-3} mol/l) in THF

B: a solution of **1-Zn** (3.0 x 10^{-3} mol/l) and ATP-Na$_2$ (1.0 x 10^{-3}, 2.0 x 10^{-3}, 4.0 x 10^{-3},

8.0 x 10^{-3} mol/l)

C: a solution of H_2O_2 (0.2 mol/l) and sodium carbonate (5.0×10^{-3} mol/l)

For a typical run, the solution of C (0.5 ml) was added to the mixture of the solution A (0.5 ml) and the solution B (1.0 ml), and the photons generated were measured for 10 seconds.

Acknowledgements

References

[1] L. You, D. Zha, E. V. Anslyn, Recent advances in supramolecular analytical chemistry using optical sensing, Chem. Rev.115 (2015) 7840-7892.

[2] A. P. de Silva, H. Q. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, Signaling recognition events with fluorescent sensors and switches, Chem. Rev. 97 (1997) 1515-1566.

[3] A. E. Hargrove, S. Nieto, T. Zhang, J. L. Sessler, E. V. Anslyn, Artificial receptors for the recognition of phosphorylated molecule, Chem. Rev. 111 (2011) 6603-6782.

[4] L. Wang, L. Yuan, X. Zeng, J. Peng, Y. Ni, J. Cheng Er, W. Xu, B. K. Agrawalla, D. Su, B. Kim, Y. –T. Chang, Multisite-binding switchable fluorescent probe for monitoring mitochondrial ATP Level fluctuation in live cells, Angew. Chem. Int. Ed. 55 (2016) 1773-1776.

[5] J.- H. Zhu, C. Yu, Y. Chen, J. Shin, Q. –Y. Cao, J. S. Kim, A self-assembled amphiphilic imidazolium-based ATP probe, Chem. Commun. 53 (2017) 4342-4345.

[6] A. Ojida, Y. Mito-oka, M. Inoue, I. Hamachi, First artificial receptors and chemosensors toward phosphorylated peptide in aqueous solution, J. Am. Chem. Soc. 124 (2002) 6256-6258.

[7] H. Tamamura, A. Ojida, T. Ogawa, H. Tsutsumi, H. Masuno, H. Nakashima, N. Yamamoto, I. Hamachi, N. Fujii, Identification of a new class of low molecular weight antagonists against the chemokine receptor CXCR4 faving the dipicolylamine-zinc(II) complex structure, J. Med. Chem. 49 (2006) 3412-3415.

[8] R. J. Wandell, A. H. Younes, L. Zhu, Metal-coordination-mediated sequential chelation-enhanced fluorescence (CHEF) and fluorescence resonance energy transfer (FRET) in a heteroditopic ligand, New J. Chem. 34 (2010) 2176-2182.

[9] D. S. Kopchuk, A. M. Prokhorov, P. A. Slepukhin, D. N. Kozhevnikoov, Design of ICT-PET fluorescent probes for zinc(II) based on 5-aryl-2,2'-bipyridines, Tetrahedron Lett. 53 (2012) 6265-6268.

[10] S. L. Murov, in Handbook of Photochemistry, Marcel Dekker Inc. New York, 1973, p.3.

[11] S. Nakatsuji, K. Matsuda, Y. Uesugi, K. Nakashima, S. Akiyama, G. Katzer, W. Fabian, Synthesis and absorption/emission spectral properties of styrylstilbene and distyrylanthracene Derivatives, J. Chem. Soc. Perkin Trans. 2, (1991) 861-867.

[12] J. Motoyoshiya, N. Sakai, M. Imai, Y. Yamaguchi, R. Koike, Y. Takaguchi, H. Aoyama, Peroxyoxalate chemiluminescence of N,N'-bistosyl-1H,4H-quinoxaline-2,3-dione and related compounds. Dependence on electronic nature of fluorophores, J. Org. Chem. 67 (2002) 7314-7318.

[13] T. W. Campbell, R. N. McDonald, Synthesis of hydrocarbon derivatives by the Wittig synthesis. I. Distyrylbenzenes, J. Org. Chem. 24 (1959) 1246-1251.

[14] H. N. Lee, K. M. K. Swamy, S. K. Kim, J. -Y. Kwon, Y. Kim, S. -J. Kim, Y. J. Yoon, J. Yoon, Simple but effective way to sense pyrophosphate and inorganic phosphate by fluorescence changes, Org. Lett. 9 (2007) 243-246.

[15] A. Ojida, Y. Mito-oka, K. Sada, I. Hamachi, Molecular recognition and fluorescence sensing of monophosphorylated peptides in aqueous solution by bis(zinc(II)-dipicolylamine)-based artificial receptors, J. Am. Chem. Soc. 126 (2004) 2454-2463.

[16] K. Kikuchi, Design, synthesis and biological application of chemical probes for bio-imaging, Chem. Soc. Rev. 39 (2010) 2048-2053.

[17] K. M. Dean, A. E. Palmer, Advances in fluorescence labeling strategies for dynamic cellular imaging, Nat. Chem. Biol. 10 (2014) 512-523.

[18] M. H.-Roberts, M. Wainwright, RW. Horobin, Real-time imaging of bacteria in living mice using a fluorescent dye, Biotech. & Histochem. 86 (2011) 104-107.

[19] W. H. Laarhove, Th. J. H. M. Cuppen, R. J. F. Nivard, Photodehydrocyclizations in stilbene-like compounds. II. Photochemistry of distyrylbenzenes, Tetrahedron, 26 (1976) 1069-1083.

[20] P. Lechtken, N. J. Turro, Peroxyloxalate chemiluminescence. Chemiexcitation of high-energy excited states in acceptor molecules, Mol. Photochem. 6 (1974) 95-99.

[21] M. M. Rauhut, Chemiluminescence from concerted peroxide decomposition reactions Acc. Chem. Res. 2 (1969) 80-87.

[22] L. F. M. Ciscato, F. A. Augusto, D. Weiss, F. H. Bartoloni, S. Albrecht,H. Branbl, T. Zimmermann, W. J. Baader, The chemiluminescent peroxyoxalate system: state of the art almost 50 years from its discovery, Arkivoc, (2012) 391-430.

[23] F. A. Augusto, A. G. de Souza, S. P. de Souza, Jr. M. Khalid, W. J. Baader, Efficiency of electron transfer initiated chemiluminescence, Photochem. Photobiol. 89 (2013) 1299-1317.

[24] R. Koike, J. Motoyoshiya, Y. Takaguchi, H. Aoyama, The key intermediates that interact with the fluorophores in the peroxyoxalate chemiluminescence reaction of 2,4,6-trichlorophenyl N-aryl-N-tosyloxamates, Chem. Commun. (2003) 794-795.

[25] T. Maruyama, S. Narita, J. Motoyoshiya, The Hammett correlation between distyrylbenzene substituents and chemiluminescence efficiency providing various ρ-values for peroxyoxalate chemiluminescence of several oxalates, Photochem. Photobiol. A: Chem. 252 (2013) 222-231.

[26] C. V. Stevani, S. M. Silva, W. J. Baader, Studies on the mechanism of the excitation step in peroxyoxalate chemiluminescence, Eur. J. Org. Chem. (2000) 4037-4046.

[27] J. Lee, H. H. Seliger, Quantum yields of the luminol chemiluminescence reaction in aqueous and aprotic solvents, Photochem. Photobiol. 15 (1972) 227-237.