Chlorophyll Chemiluminescence Emitted by Chlorin Ring Cleavage with Acetonitrile and Hydrogen Peroxide

Toshimasa Nagoshi and Shukuro Igarashi
Department of Materials Science, Faculty of Engineering, Ibaraki University, Nakanarusawa, Hitachi 316-8511

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In the presence of acetonitrile and NaOH, when H_2O_2 was added to the solution containing chlorophyll, it was found that a strong chemiluminescence (CL) was emitted. The blue light was emitted with the decomposition of chlorophyll. It is considered that the emitter contains the decomposition products of chlorophyll based on the following measurement results, such as the reduction of absorbance for chlorophyll and the difference of the spectral form between fluorescence and CL. The CL of the chlorines, such as chlorophyll, and the porphyrin analogue compounds were compared under the same conditions. Consequently, the obtained CL was strong in the chlorin complex which contains Mg or Zn. On the other hand, the CL was not strongly observed with porphyrin compounds. In the chlorophyll CL, when the chlorin ring is oxidized by the generated $\cdot O_2^-$, the excited molecule would be produced in the process of ring cleavage, and then CL is emitted to the outside.

CL is the phenomenon in which light is emitted by a chemical reaction, and CL is not only used for determining bloodstains or chemical light, but is also applied to the ultratrace analysis of metal ions, enzymes, DNA, etc.[1,2] The principle of luminescence is based on the fact that a molecule as an emitter becomes an excited state by the chemical reaction, and then the light is emitted when it returns to the ground state. Most CL reactions proceed by the generation and cleavage of dioxetane.[3,4] More applications are expected in the field of ultratrace analysis using CL, because CL takes advantage of its sensitivity since CL does not need an excitation light source. However, there are only about ten kinds of CL reagents that emit a strong light. For example, luminol, lucigenine, acridinium ester, rofin, indole, xanthene, and phenanthroline. As others, there is siloxene in which a carbon atom is not involved. Moreover, there is pyrogallol CL, whose emitter is singlet oxygen, and peroxyoxalate CL that occurs by the energy transfer to fluorecent compounds. Therefore, even today most reports of CL are based on the above CL reactions, so the discovery of a new CL reagent would be very important for expansion of this technique. Hence, the authors searched for a new CL reagent centered around compounds that have a porphyrin and chlorin ring. As a result of this study, it was found that a strong CL was emitted from a Mg–, Zn–chlorin complex, and especially chlorophyll.

The obtained CL signal is shown in Figure 1, when H_2O_2 was added to the solution containing chlorophyll a in the presence of acetonitrile and NaOH. The concentration of chlorophyll a was only 18 ng/mL. The absorption spectrum in the reaction system was observed after adding the H_2O_2. As a result, the both absorbance at the Soret band and Q band of chlorophyll a decreased for a short time. The reduction speed of the absorbance was mostly in agreement with the increase and reduction in the CL signal.

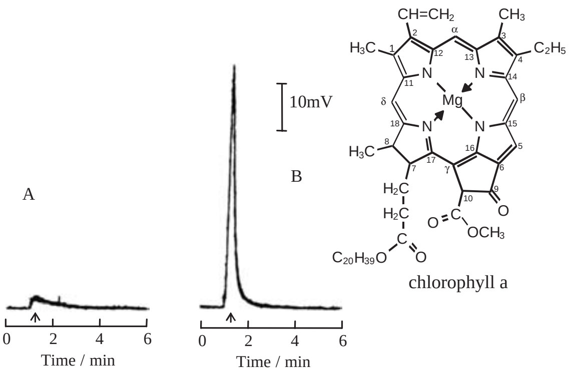

Figure 1. Typical CL signal in the chlorophyll CL reaction. A: 0 ng/mL chlorophyll a, B: 18 ng/mL chlorophyll a, $[H_2O_2]_T = 0.13$ M, acetonitorile 34%, $[NaOH]_T = 3.4 \times 10^{-4}$ M, room temp.

The fluorescent spectrum change at the excitation wavelength of 415 nm (Soret band) and the emitted CL spectrum of this reaction are shown in Figure 2. By comparing each spectrum, the maximum wavelength shifted 25 nm to the blue side in the CL spectrum. However, this broad CL spectrum may be produced due to the mixture of unreactive chlorophyll and decomposition products. In the near future, this point would be clarified. Based on this fact, it can be expected that the emitter produced in this reaction is not only the chlorophyll itself but also its decomposition products. The singlet oxygen ($^1\Delta g + {}^1\Sigma g^+$, $2{}^1\Delta g$), otherwise considered to be the emitter, differs from it since the maximum wavelengths of the luminescence spectrum are 478 and 637 nm, respectively.[2]

Table 1 indicates the result of comparing the various chlorins that are the main ring of chlorophyll and the analogue porphyrin under the same conditions. A strong CL was emitted from chlorophyll a and chlorophyll b. In the case of pheophytin a and

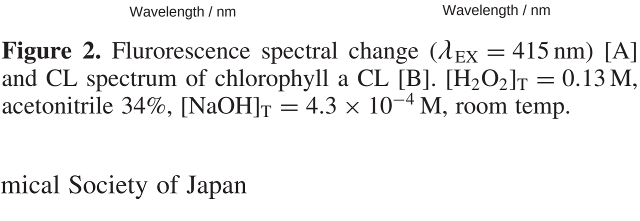

Figure 2. Flurorescence spectral change ($\lambda_{EX} = 415$ nm) [A] and CL spectrum of chlorophyll a CL [B]. $[H_2O_2]_T = 0.13$ M, acetonitrile 34%, $[NaOH]_T = 4.3 \times 10^{-4}$ M, room temp.

Table 1. Comparison of CL intensity from chlorine and porphyrin analogue

	CL Intensity		CL Intensity
None	3.1	uroporphyrin	10.0
Chlorophyll a	9510.0	TPP	2.2
Chlorophyll b	9530.0	TCPP	18.8
Pheophytin a	88.3	THPP	2.2
Pheopholbate a	99.0	TSPP	3.0
Chlorin e_6	250.0	Mg-TSPP	1.6
Zn–Chlorin e_6	2530.0	TMPyP	4.1
Coproporphyrin	5.2	bilirubin	13.3

$[\text{chlorin or porphyrin analogue}]_T = 5 \times 10^{-6}$ M, $[H_2O_2]_T = 0.13$ M, acetonitrile 34%, $[NaOH]_T = 4.3 \times 10^{-4}$ M, room temp.,
TCPP: 5,10,15,20-tetrakis(4-carboxyphenyl)porphine,
THPP: 5,10,15,20-tetrakis(4-hydroxyphenyl)porphine,
TSPP: 5,10,15,20-tetrakis(4-sulfophenyl)porphine,
TMPyP: 5,10,15,20-tetrakis(*N*-trimethylaminiophenyl)porphine.

pheopholbite a, which do not have Mg at the center, only about 1/100 of the CL intensity was emitted as compared with the chlorophyll. On the other hand, chlorin e_6 emitted a little light, and its Zn complex showed a strong CL. Furthermore, certain kinds of porphyrin (coproporphyrin, uroporphyrin, TPP, TCPP, THPP, TSPP, Mg-TSPP, TMPyP) and bilirubin, whose structure has an opened chlorin ring, hardly emitted light under the same conditions. And these porphyrins did not decompose in the same conditions.

Three points can be considered as a reason that this CL reaction is specific to chlorophyll. One is because chlorophyll has the chlorin ring. The chlorin ring is lower than the porphyrin ring in the points of resonance stability since it lacks a double bond as compared with the porphyrin ring and the spread of the resonance system is low. Therefore, it is easy to undergo oxidation by H_2O_2.[5] The 2nd reason is expected that the fluorescence intensity and fluorescent quantum yield of the emitter increase with Mg that is the central metal of chlorophyll. When chlorin or the porphyrin complex contain Mg and Zn, their fluorescent intensity increase. In fact, the fluorescent intensity of chlorophyll a is about 3 times greater when compared with pheophytin a (the case of acetone solvent). In this reaction system, since the decomposition products from chlorophyll are also expected to be emitters, consequently, it would be a large difference in the CL intensity. For the 3rd reason, the influence of the positive electric charge of Mg can be considered. Since it is reported that the strength of the positive electric charge on the central part correlates with the interaction between chlorophyll and an oxygen molecule, the reactivity with $\cdot O_2^-$ produced from H_2O_2 similarly becomes large for the Mg and Zn complex in this reaction system. Therefore, the reaction rate is considered to quickly increase.[6] Actually, the appearance time of the CL signal peak differed between chlorophyll a and pheophytin a. (chlorophyll a = 25 s, pheophytin a = 60 s, under the same conditions in Table 1).

Table 2. Effect of reaction media for chlorophyll CL

Polar Solvent	CL Intensity
Methanol	2.2
Ethanol	2.8
t-Butanol	3.3
Acetone	2.0
Acetonitrile	81.2
Dimethyl solfoxide	8.3
N,*N*-Dimethylformamide	2.4
Tetrahydrofuran	2.1

$[\text{chlorophyll a}]_T = 3 \times 10^{-8}$ M, $[H_2O_2]_T = 0.13$ M, polar solvent 34%, $[NaOH]_T = 4.3 \times 10^{-4}$ M, room temp.

Table 2 shows the effect of the reaction media on the chlorophyll CL. In this study, a strong CL was only emitted in acetonitrile. It was reported that $\cdot O_2^-$ was formed from the coexistence of acetonitrile and H_2O_2.[7] When super oxide dismutase (SOD) was added to the chlorophyll CL, the CL intensity decreased. This shows the formation of $\cdot O_2^-$ and it can be expected that a certain dioxetane structure was formed and the excited cleavage molecule emits light like the other CL reactions.

In conclusion, the CL reaction, which we found, was a new CL reaction in which chlorophyll fulfills the function as the photogen. This CL reaction can be applied to the measurement of ultratrace amounts of chlorophyll in environmental water, foods, and medicines. Additionally, this would be of interest not only in analytical chemistry but also in the field of biochemistry, etc., since chlorophyll is the activity center of photosynthesis in plants.

References

1 S. Igarashi, T. Nagoshi, and T. Kotake, *Anal. Lett.*, **33**, 3571 (2000).
2 "Bioluminescence and Chemiluminescence" ed. by K. Imai, Hirokawashoten, Tokyo (1988), Chap. 4.
3 F. McCapra, I. Beheshti, A. Burford, R. A. Hann, and K. A. Zaklika, *J. Chem. Soc., Chem. Commun.*, **1977**, 944.
4 T. Goto, *J. Synth. Org. Chem. Jpn.*, **37**, 275 (1979).
5 K. M. Smith, S. B. Brown, R. F. Troxler, and J. Lai, *Tetrahedron Lett.*, **21**, 2763 (1980).
6 T. Nishikawa, *Bunseki Kagaku*, **38**, 182 (1989).
7 G. B. Payne, P. H. Deming, and P. H. Williams, *J. Org. Chem.*, **26**, 659 (1961).

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