Journal of Luminescence 165 (2015) 15–18

Contents lists available at ScienceDirect

Journal of Luminescence

journal homepage: www.elsevier.com/locate/jlumin

Effect of very high magnetic field on the optical properties of firefly light emitter oxyluciferin

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ARTICLE INFO

Article history:
Received 29 January 2015
Received in revised form
12 April 2015
Accepted 13 April 2015
Available online 23 April 2015

Keywords:
Firefly
Bioluminescence
Oxyluciferin
Magnetic field
Photoluminescence

ABSTRACT

Magnetic field effect on enzymatic reactions is under intensive study in the past decades. Recently, it was reported that firefly bioluminescence was suppressed and red-shifted significantly when exposed to external magnetic field. However in this work, by means of selective excitation, we confirmed that emission properties of firefly light emitter "oxyluciferin" are completely immune to external magnetic field of up to 53 T. These findings pose strong contrast to existing relevant results. Potential reasons for the discrepancies found and the underlying physics towards the understanding of firefly bioluminescence were discussed.

1. Introduction

Magnetic field effect on enzymatic reactions is a subject of extensive study in the past decades [1–3], as human beings and other living creatures are exposed to all kinds of magnetic fields more and more frequently. Understanding and controlling enzymatic reactions are not only fundamentally interesting, but also practically important. Recently, it was reported that the emission of light by a living creature, *i.e.* bioluminescence, is affected by external magnetic field significantly [4]. Iwasaka and his co-workers demonstrated, using living fireflies, that firefly bioluminescence was suppressed in intensity and red-shifted for ~10 nm when exposed to a 14 T magnetic field. As an ideal "cold light" system with extremely high quantum yield of up to 41.0 ± 7.4% [5], firefly bioluminescence lies in the center of biochemistry research [6–19]. The revealing of magnetic field effects on firefly bioluminescence therefore represents significant progress in this field. However, as living fireflies were used in their work, there are concerns regarding the accuracy of the spectroscopy measurement. Furthermore, effects of much higher magnetic fields on firefly bioluminescence, as well as the mechanism for magnetic field induced spectral changes, remain unclear so far. Further work is needed to clarify these issues.

In this work, we report high-field magneto-optical study on the emission properties of firefly light emitter oxyluciferin, aiming to clarify the effects of high magnetic field on enzymatic reactions and the underlying mechanism. Unlike in the work by Iwasaka and his co-workers, we employed *in vitro* luciferin–luciferase reaction, instead of living fireflies, to increase the stability and reproducibility in the experiment and thus the experimental accuracy could be improved greatly.

As a result of the efforts devoted, it is nowadays clear, that firefly bioluminescence results from the oxidation process of the substrate molecule luciferin, with the help of the enzyme luciferase [5,7,20–30]. As illustrated in Fig. 1, firefly bioluminescence is known to be a multi-step process. In the first step, the substrate luciferin is oxidized by molecular oxygen at the luciferase active site, with adenosine-5′-triphosphate (ATP) and magnesium ion as cofactors, and generates a high energy intermediate product dioxetanone. Dioxetanone, whose decomposition mechanism is under intensive study [31–33], is unstable. It decomposes and generates the final product oxyluciferin in an electronically excited state. Photon emission, *i.e.* bioluminescence, happens when the electronically excited oxyluciferin relaxes to its ground state. From these reaction steps, one could see clearly, that oxyluciferin is the real light emitter in firefly bioluminescence. Therefore, photoluminescence (PL) from oxyluciferin is essentially a close analog to firefly bioluminescence. Moreover, compared with bioluminescence, PL from oxyluciferin gives continuous and stable

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http://dx.doi.org/10.1016/j.jlumin.2015.04.009
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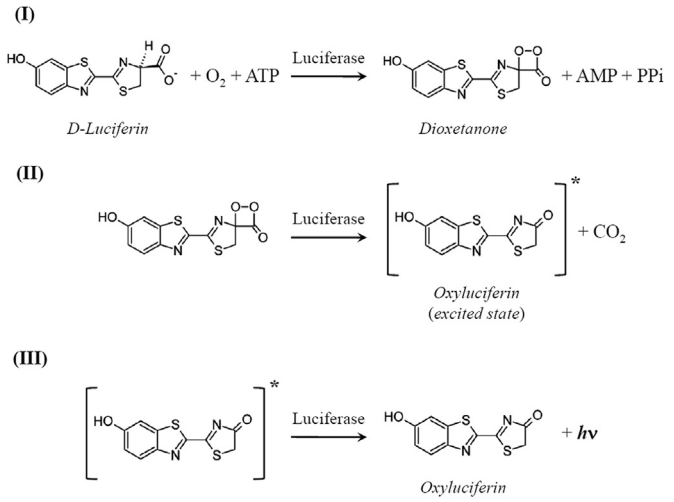

Fig. 1. Reaction mechanism of the luciferase-catalyzed firefly bioluminescence summarized into three steps. The final reaction product oxyluciferin has six possible forms and presented here in its neutral keto form. Symbol "*" in Steps II and III represent electronically excited state of oxyluciferin.

emission as long as external pumping is on. It could thus facilitate a much more reliable optical measurement.

2. Experimental

On the other hand, to simulate the micro-environment of firefly bioluminescence, the oxyluciferin molecules used in this work were generated directly from the luciferin–luciferase reaction. The enzyme we used is *Luciola cruciata* (*Lcr*) luciferase recombinated from *Escherichaia coli*. The activity and bioluminescent reaction catalyzed by this enzyme have been confirmed to be the same as the wild type luciferase in our previous work [34]. GTA buffer solution (0.15 M, containing 0.05 M 3,3-dimethylglutaric acid, 0.05 M 2-amino-2-hydroxymethyl-1,3-protanediol, and 0.05 M 2-amino-2-methyl-1,3-propanediol) was used to dilute the reactants to the expected concentrations. The concentration of the D-luciferin was estimated to be 4.0×10^{-8} M. The *Lcr* luciferase solution of the estimated concentration of 8.0×10^{-6} M was prepared by dissolving the luciferase powder with the GTA buffer solution containing 10% glycerol. The pH of the reaction solution was ~6.6. The concentration of the luciferase was made much higher than that of the substrate luciferin to ensure that all luciferin molecules can be consumed in the luciferin–luciferase reaction. The *in situ* PL measurement was performed right after the luciferin–luciferase reaction completed. All measurements were finished within one hour after the reaction, during which the reaction product was stable [35].

For magneto-optical measurement, the pulsed high magnetic field was generated by a home-made wire-wound solenoid coil with maximum field of ~53 T and pulse duration time of ~40 ms [36,37]. The excitation laser beam and the photoluminescence signal from oxyluciferin were both guided by optical fibers. All magneto-photoluminescence spectra were taken on the top of the pulsed magnetic field with detector gate opening time of ~5 ms, during which the field variation is maintained within 4%.

3. Results and discussion

Typical bioluminescence spectrum of the *Lcr* fireflies (300 K, pH ~6.6) is shown in Fig. 2(a). A broad peak, centering around 2.1 eV, can be clearly observed. However, the spectrum is highly asymmetric

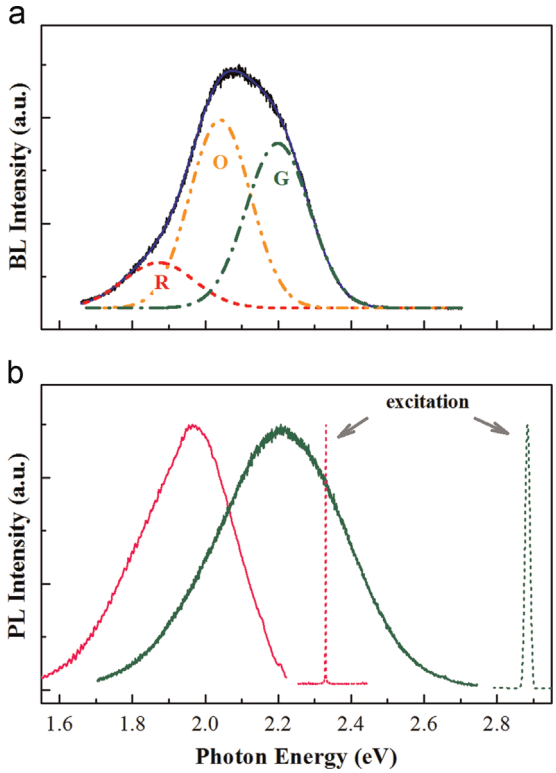

Fig. 2. (a) Spectrum of *Luciola cruciata* firefly bioluminescence at room temperature. Together shown in the figure are the three Gaussian components that reproduce the spectrum. Green dash-dotted line: pH-sensitive green component at ~2.2 eV (Peak "G"); and orange dash-dot-dotted and red dashed lines: pH-insensitive orange and red components at ~2.0 eV (Peak "O") and ~1.88 eV (Peak "R"), respectively. pH of the reaction solution: ~6.6. (b) *In situ* photoluminescence of the light emitter oxyluciferin ($OxyLH_2$) in complex with luciferase. Green line: photoluminescence with excitation at 430 nm (selected from a halogen lamp by monochromator); pink line: photoluminescence excited by laser at 532 nm; green dashed line: excitation light at 430 nm; and pink dashed line: excitation light at 532 nm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in its lineshape, implying strongly that it consists of several components that probably correspond to different species of the oxyluciferin. This issue has been clarified in our previous work using quantitative spectroscopy [5,34]. In Refs. [5] and [34], our quantitative spectroscopy measurements reveal that firefly bioluminescence spectrum can be systematically decomposed into three Gaussian components, which locate in the green, orange and red spectral regions, respectively. To compare with these existing results, we also carried out curve fittings using three Gaussian profiles, the same as we did in Refs. [5] and [34]. As expected, the bioluminescence spectrum is well reproduced by three Gaussian peaks locating at ~2.2, ~2.0 and ~1.88 eV, respectively, as shown in Fig. 2(a).

To find out the effects of high magnetic field on firefly bioluminescence and the underlying mechanism, one efficient way is to study how these three Gaussian components respond to the external magnetic field. Fortunately, it is nowadays clear that these three Gaussian components originate from different species of the light emitter oxyluciferin and can be selectively excited [10,20–22,35]. Typical *in situ* PL spectra of the oxyluciferin in complex with luciferase in the reaction mixture are shown in Fig. 2(b). The fluorescence spectra with excitation at 430 nm (~2.88 eV, green curve) and 532 nm (~2.33 eV, pink curve) peak at ~2.2 eV and ~1.97 eV, respectively. Compared with the Gaussian fittings of the bioluminescence spectrum, it can be seen clearly, that the photoluminescence with 430 nm excitation (green curve) resembles closely the green component in the bioluminescent spectrum (Peak "G", Fig. 2(a)). On the other hand, the PL spectrum with excitation at 532 nm (pink