Spectral properties of Lampyris Noctiluca firefly from Sumatra

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Abstract. One type of creeping firefly (Lampyris noctiluca) has been found in the forest area, West Sumatra, Indonesia. This study reports the spectral properties of these fireflies using a nanofotometer. From the measurement results, the wavelength value at maximum intensity is 580 nm. The decay constant, the number of photons, the quantum yield and the activation energy produced are 0.0092 / sec, 4.95 quanta / sec, 0.283 and 2.1433 eV, respectively.

1. Introduction

The bioluminescence phenomenon of fireflies is still attracting the attention of scientists. Bioluminescent of fireflies have various colors, patterns, periodic, and light intensities. This light is used by fireflies for self defense against predators, camouflage, intra-specific communication, and attract mates or prey. The application of light fireflies spread to various fields such as gene expression, protein interactions, disease development and recombinant biosensors throughout cells [1][2][3].

Fireflies can emit light caused by chemical reactions involving three main components, namely luciferin (substrate), luciferase (enzyme) and oxygen molecules [4][5]. Luciferin is a substrate that opposes heat and produces light and luciferase is an enzyme that catalyzes and oxygen as fuel.

The bioluminescence spectral properties of many firefly species differ from one another. This spectral characteristic is influenced by many factors including the environmental conditions where the fireflies are located [5]. Fireflies of the Luciola Praeusta species from India have wavelengths at optimum intensity at 562 nm [6]. Lampyris noctiluca species from North America have wavelengths at maximum intensity at 555 nm [7]. Creeping species of Lamprophorus sp from the West Sumatra region have a maximum wavelength of 540 nm. The same wavelength is obtained for Pteroptyx tener flying fireflies which also originate from West Sumatra [8]. Other spectral characteristics such as the relative quantum yield value and the number of photons produced are different. For the creeping fireflies (Lamprophorus sp), the relative quantum yield value is 153,043 Light Units and the emitted photons are 7.04 x 10^11 quanta / sec, the relative yield and number of photons produced from the flying fireflies (Pteroptyx tener) is 215,913 light it and 9.89 x 10^11 quanta / second, respectively.

Furthermore, found a firefly creeping from the species Lampyris noctiluca from the West Sumatra region. Spectral properties of these fireflies have not been reported yet. This article reports the spectral properties of the Lampyris noctiluca fireflies such as wavelengths at maximum intensity, decay constants, number of photons emitted, quantum yield and activation energy.
2. Methods

2.1. Sample Preparation
The sample of used was fireflies that had been isolated. Submerge the fireflies in liquid nitrogen for 10 minutes. Cut and mash the glowing parts of the fireflies. add 2 mL acetone, add 5 mL buffer solution then stir until mixed. Soaking the sample in an ice cube. Then setrifugation with a speed of 10,000 rpm with a temperature of 4°C for 10 minutes. Then filter the supernatant.

2.2. Measurement
Prepare a sample. Turn on the nanofotometer, then insert the firefly extract plus ATP into the nanofotometer cuvette. Take measurements of wavelength and relative intensity of fireflies by varying the time. To determine the decay constant, the quantum yield and the number of photons emitted per unit of time is done by measuring the maximum relative intensity at the maximum wavelength of each time change. Calculates the intensity at wavelength with the maximum relative intensity at each time change. Data processing techniques use the following equation:

2.2.1. Determine Decay constant

\[
\log I = \log I_0 - \frac{k}{2.303} t
\]

\(I_0\) is initial intensity of light
\(I\) is final intensity of light
\(k\) is decay constant
\(t\) is decay time.

2.2.2. Determine the relative Quantum yield

\[cq = \frac{I_0}{k}\]

2.2.3. Determine the number of photons

\[N = cq = \int_0^\infty I dt\]

2.2.4. Determine the activation energy

\[E_a = h\nu = \frac{hc}{\lambda} \left(1.6 \times 10^{-19}\right)\]

3. Results and Discussion
Measurement of wavelengths with the relative intensity of creeping fireflies of the Lampyris noctiluca species, shown in Figure 1.
Figure 1. Bioluminescence wavelength spectrum of creeping fireflies of the Lampyris noctiluca species

Figure 1 shows that the wavelength at the maximum intensity of the creeping fireflies of the Lampyris noctiluca species is 580 nm. Furthermore, from the measurement results can be plotted the relationship of log I to the decay time t, as shown in Figure 2.

Figure 2. Relationship of Intensity to Log I to the decay time t with a wavelength of 580 nm.

From Figure 2 we can calculate the decay constant k using equation (1) and the value is 0.0092 / s. Furthermore, by using equation (2), the resulting photon value is 107.682 light units. Furthermore, by using the press. (3) relative quantum yield can be calculated. Quantum yield in a reaction in the light emitting fireflies is defined as the amount of quanta emitted by the reaction permolecule. The resulting quantum yield is 21.53 x 1014 quanta /s.mg. Because 1 mg molecule consists of 7.6 x 1015 molecules, the quantum yield obtained from the light emitting fireflies is 0.283. The number of photons (N) per second unit emitted by fireflies can be calculated in the amount of 4.95 x 1014 quanta / second. By using equation (4), the amount of activation energy (Ea) is obtained, which is 2.1433 eV.

Comparison of experimental results about physical characteristics such as wavelength (λemission), decay constant (k), quantum yield (q), number of photons (N) and the Activation Energy of light emitting on light fireflies Lampyris noctiluca with fireflies others can be seen in Table 1.
Table 1. Comparison of physical characteristics of light emitting creeping fireflies from Lampyris noctiluca species with other fireflies

| Firefly               | λ (nm) | k     | Q   | N(quanta/s)  | Ea (eV) |
|----------------------|--------|-------|-----|--------------|---------|
| Lampyris noctiluca   | 580    | 0.0092| 0.283| $4.95 \times 10^{14}$ | 2.1433  |
| Firefly [9]          | 560    | -     | 0.88| -            | 2.219   |
| Firefly [10]         | -      | -     | 0.9 | -            | -       |
| Pteroptyx tener [11] | 540    | 0.0046| 215,913| $9.89 \times 10^{11}$ | 2.302   |

Table 1 can be concluded that the spectral properties of bioluminescence in fireflies have differences with other fireflies. The difference lies in the wavelength of the emission, the quantum yield, the number of photons and the Activation Energy produced. This difference is due to one originating from the characteristics of the environment where the fireflies are found. Environmental characteristics that influence are such as temperature, pH, soil, food and others. Therefore it is not surprising that many fireflies were reported by scientists to have different spectral properties. It can be understood that the wavelengths of light produced by fireflies range from 510 to 670 nm with a pale color from yellow to reddish green.

4. Summary
In Summary, the maximum wavelength obtained from fireflies is 580 nm. The decay constant, the number of photons, the quantum yield and the activation energy produced are 0.0092 / sec, 4.95 quanta/sec, 0.283 and 2.1433 eV, respectively. Physical characteristics of light emitting on fireflies have differences with the physical characteristics of light emitting on other fireflies. The difference lies in the wavelength of the emission, the quantum yield, the number of photons and the Activation Energy produced even though the structure of the enzyme and substrate is the same. This difference is due to the characteristics of the place and the fireflies are found.

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References
[1] Dikmen Z.G, Gellert G, Dogan P, and Mason R 2005 Turk J Med Sci 35 (2) pp. 65-70.
[2] J.G. Bundy J.G, Wardell J.L, Campbell C.D, Killham K, and Paton G. 1997 Letters in Applied Microbiology 25(5) pp. 353–58.
[3] Griffiths, M.W 2010 Journal of Dairy Science 76(10) pp. 3118–25.
[4] Bay A and Vigneron J.P 2009 Proceedings of SPIE 7401(0) pp. 740108-740108–12.
[5] Min C G, Ren A.M, Guo J.F, Zou L.Y, Goddard J.D, and Sun C.C 2010 ChemPhysChem 11 pp. 2199–2204.
[6] Barua A, Gohain S, Hazarika N.M, Saikia and Baruah G.D 2009 J. Biosci 34 pp. 287–292.
[7] David B, Alan J. A, Stewart and Osorio D 2004 The Journal of Experimental Biology 207 pp. 2373-2378.
[8] Ratnawulan R. Fauzi A. Arma A.S 2019 IOP Conf. Series: Journal of Physics: Conf. Series 1185 p.012016
[9] Li Y 1999 School of Electrical and Electronic Engineering Nayang Technological University 76
[10] Brovko L 2007 Bioluminescence for food and environmental microbiological safety. U.S.A.: SPIE.
[11] Sari M, Ratnawulan R, and Gusnedi 2014 Pillar Of Physics 1 pp. 113-120.