Sharp intense line in the bioluminescence emission of the firefly

A. Gohain Barua · U. Sharma · M. Phukan · S. Hazarika

Received: 30 December 2013 / Accepted: 5 March 2014 / Published online: 26 April 2014
© Springer Science+Business Media Dordrecht 2014

Abstract Numerous investigations have been carried out on the spectral distribution of the light of different species of fireflies. Here we record the emission spectrum of the Indian species of the firefly *Luciola praeusta* Kiesenwetter 1874 (Coleoptera : Lampyridae : Luciolinae) on a color film. Green and red color-sectors, with an intense yellow one in between, appear in this spectrum. Intensity profile of this spectrum reveals a hitherto undetected strong narrow yellow line, which lies within the full-width-at-half maximum (FWHM) of the intensity profile. The spectrum recorded in a high-resolution spectrometer confirms the presence of this sharp intense line. This finding lends support to an earlier drawn analogy between the *in vivo* emission of the firefly and laser light.

Keywords Firefly light · Emission band · Sharp yellow line · Random laser

1 Introduction

The process by which living organisms convert chemical energy into light is called bioluminescence. Firefly bioluminescence is well-known for its extremely high quantum yield value [1–3]. In this light-producing cold system, the chemiluminescence reaction, catalyzed by the enzyme luciferase, uses luciferin, O_2 , ATP and Mg^{2+} to yield an electronically excited oxyluciferin species. Visible light is emitted as the oxyluciferin decays to the ground state.

The light of the firefly has been generating considerable interest among naturalists and biochemists due to the complicated chemical reactions involved, as well as among electro-optical physicists for the objective of generating laser light by efficient chemical means. Also, it is now considered that the modification of firefly bioluminescence by utilizing magnetic fields could potentially open a new horizon.

Spectral distributions of firefly bioluminescence have been studied for over a century. The two smallest values of full-width-at-half-maximum (FWHM), reported by Coblenz in

A. Gohain Barua (✉) · U. Sharma · M. Phukan · S. Hazarika
Department of Physics, Gauhati University, Guwahati 781014, India
e-mail: agohainbarua@yahoo.com

1912 using photographic spectrometry, are 33.3 and 46.7 nm for the North American species *Photinus pyralis* and *Photinus consanguineus* [4]. If we leave aside these two values, being exceedingly small, the narrowest half width reported is 55 nm for the Indian species *Luciola praeusta* [5]. Approximately equal *in vitro* FWHM values have been reported, for example, in the green-emitting luciferase of the Japanese firefly *Pyrocoelia miyako* [6]. There have been reports of the existence of distinct groups of bands in a few species of fireflies [7–9]. It has been suggested that different species of fireflies emit in slightly different spectral regions due to slight differences in enzyme structures [10]. An investigation on the structural basis for the spectral difference in luciferase bioluminescence has indicated that the degree of molecular rigidity of the excited state of oxyluciferin, which is controlled by a transient movement of Ile 288, determines the color of bioluminescence during the emission reaction [11]. Recording colored spectra, it is hypothesized that though fireflies belonging to the species *Luciola praeusta* emit a substantial part of the radiation in the red sector of the spectrum, the red color is not visible to the naked eye because of the Purkinje effect, and it is inferred that the firefly light has a tendency for spectral narrowing in the narrow yellow region [12]. The bioluminescence spectra of firefly luciferases (pH-sensitive) have been long shown to undergo red shift at lower pHs and other denaturant conditions [13]. Recently, it was observed that the bioluminescence spectrum of the Japanese species *Luciola lateralis* shows a redshift under a strong static magnetic field [14].

2 Materials and methods

A few adult male specimens of the firefly species *Luciola praeusta* Kiesenwetter 1874 (Coleoptera : Lampyridae : Luciolinae) were caught in the Gauhati University campus in the early evening hours just prior to the experiment. Those were made to inhale vapors of ethyl acetate in the manner described elsewhere [5]. After about six minutes, continuous glows were seen from their abdominal light-producing organs. The specimen exhibiting the brightest glow was taken for the experiment. An Ocean Optics HR2000 Series high-resolution fiber optic spectrometer had been calibrated just before the experiment with standard lines obtained from an iron arc. The anaesthetized firefly was kept in a cotton plug with its light organ positioned towards the entrance face of the fiber. After recording its emission spectrum, the firefly was taken out and fixed with cotton and cello tape at the entrance slit of a Hilger & Watts glass spectrograph. The spectrum was recorded in a 24 mm x 36 mm FUJIFILM (FUJICOLOR CRYSTAL X-TRA 400). From about 20:00 hours in the evening to about 6:00 hours the next morning, the insect was kept fitted in this way. The temperature in the laboratory during the experiment was between 26 °C and 34 °C. For reference, a spectrum from an iron arc was recorded above this one in the film. Developed positives of the colored spectra were scanned in a HP Scanjet G4010 scanner at 2400 DPI. Intensity profiles of the scanned spectra were plotted with the help of the software ImageJ.

3 Results and discussion

In the *in vivo* emission spectrum of the firefly species *Luciola praeusta*, presented in Fig. 1a, two broad green- and red-colored sectors, with a narrow yellow one in between, are clearly visible. Another noticeable aspect of this spectrum is that the yellow sector is more intense than the other two. The intensity distribution, given in Fig. 1b, clearly shows that at 591 nm the emission bandwidth narrows and the intensity increases considerably. The spectrum of

Fig. 1 Colored emission spectrum of an adult male specimen of the firefly species *Luciola praeusta* Kiesenwetter 1874 (Coleoptera : Lampyridae : Luciolinae) and its intensity profile. **a** Three colors—green, yellow and red—manifesting in the spectrum of the ‘dc’ light of the firefly recorded on a color film in a spectrograph. The red color is not visible to the naked eye under normal circumstances. **b** Intensity distribution of the recorded spectrum. A strong line with its peak at 591 nm lies in the green-red ‘border’

Fig. 2 Emission spectrum of another specimen of the species and its intensity profile. **a** The green and red sectors appearing to be more diffuse than the sharper yellow region. **b** Intensity distribution of the recorded spectrum. As in Fig. 1b, the peak at 591 nm forms a ‘divider’ between the green and red regions

another specimen presented in Fig. 2a exhibits narrower spreading of the yellow photons compared to those of the green and red ones; its intensity profile given in Fig. 2b is similar to that of Fig. 1b. This yellow line/band lies within the FWHM spread, and its intensity is

Fig. 3 Emission spectrum of the first specimen of the firefly recorded in a high-resolution spectrometer. **a** The peak wavelength appearing at 562 nm, and the full-width-at-half-maximum extending from 537 nm to 592 nm in the emission band. Values of the lower and upper half widths (HWHM), thus, come out as 25 and 30 nm, respectively. The sharp intense line at 591 nm lies within the 55 nm emission bandwidth. System noise appears as a result of keeping the integration time of the spectrometer at 3000 ms, which was necessitated by the low power of the emitted light. **b** The zero-signal spectrum. **c** The emission spectrum after subtracting the zero-signal spectrum

evidently more than that of 562 nm—the earlier reported wavelength peak in the emission spectrum of this particular species [5]. Hence this wavelength has to be treated as the peak wavelength in the spectrum. Apparently, green and red sectors form ‘discrete bands’ with the yellow line acting as a divider.

The emission spectra recorded in the high-resolution spectrometer for these two specimens, shown in Fig. 3a and Fig. 4a, respectively, bring out this narrow intense line at

Fig. 4 Emission spectrum of the second specimen of the firefly. **a** The peak, FWHM as well as the strong sharp line appearing at the same positions as those of the first specimen. **b** The spectrum in the absence of a signal. **c** The spectrum after subtracting the zero-signal one

591 nm. As a matter of fact, this line is a signal-enhanced system noise, as the noise could be easily seen at this wavelength from the no-signal spectra presented in Fig. 3b and Fig. 4b. Here no-signal means the spectrum in absence of the specimen, not in the OFF state of the firefly. Subtractions of these no-signal or zero-signal spectra from the firefly spectra, shown in Fig 3c and Fig. 4c, make the presence of the sharp line clear. It is surprising that such a line has not been detected in the spectra of any species of fireflies till now. Most probably, smoothening of the presented profiles or non-response of the detector *exactly* at this wavelength have been the reasons for this non-detection. Even in the instance of presentation without smoothening [5], this line was thought to be due to noise and consequently the ‘overshoots’ were clipped off in the spectra. There we see that the sharpness of the line appears to have been a problem. In the present case, the signal is superposed with the noise at 590.64 nm, thereby enhancing it. The narrowness of the profile is limited by the fact that our spectrometer records counts at intervals of approximately 2.2 Å. That is, the full-width-at-half-maximum (FWHM) of the line is surely narrower than those shown in Figs. 3a and 4a, which are approximately 0.25 nm or 2.5 Å—approximately equal to that of a random laser obtained by depositing zinc oxide (ZnO) powder of average particle size 100 nm onto ITO-coated glass substrates by electrophoresis [15]. It has already been observed that the time-resolved spectrum of this species of the firefly shows striking similarity with the output of a multi-mode laser [5]. A flash of duration approximately 100 ms has been seen to be composed of about 30,000 pulses—the appearance of which being the reason of drawing this analogy. It is evident from Fig. 1b and, with some difficulty, Fig. 2b that there are undershoots at the ends of the line, especially on the higher wavelength (red) side. This gives the appearance of a sinc function, the Fourier transform of a triangular function. Very recently, it is shown that pulses from an anaesthetized firefly are triangular in shape [16]. Since those pulses, representing an oscillating chemical reaction in the microsecond time scale [17], were very weak, it is reasonable to believe that those were generated by the much more energetic 591 nm yellow photons.

The intensity of the 591 nm line varies greatly vis-à-vis the earlier reported peak intensity at 562 nm. In the five spectra recorded in the spectrometer, the intensity ratio F591/F562 of these two peaks are 3.76, 3.72, 2.74, 1.57 and 1.21. Because of so much variation in the intensity ratio, it is difficult to draw a conclusion on this; suffice to say that the 591 nm peak is more intense than that of the 562 nm one. Also, the yellow line is noticeably broader in the intensity profiles of the spectra recorded on films in the spectrograph than those obtained in the spectrometer. As the exposures given in the spectrograph were for the entire durations of the nights, while those given in the spectrometer were for 3 seconds, we can hypothesize that this particular line is not emitted all the time with the flash. This could possibly be the reason for the wide fluctuation in the intensity of the yellow line.

It is well known that the firefly lantern is a slab-like tissue, consisting of a ventral photogenic layer and a dorsal reflector layer [18–21]. The photogenic layer houses the light reaction, while the reflector layer is a specialized tissue which is believed to play a key role for increasing the bioluminescence intensity through reflection. Very recently, it was reported that the cytosol of the reflector layer is filled with densely packed spherical granules, which should be uric acid granules [22]. Diameters of these granules are of the order of the wavelength of the emitted light, and it is tempting to speculate that the emitted light waves are trapped by multiple scattering by them. Thus the role of a regular laser cavity, akin to the one in the random laser [23–25], is taken over in this disordered medium. Large variation in the intensity ratio F591/F562 also points towards a mechanism like this. It could be mentioned here that in random lasers, for the particle size much larger than the emission wavelength a single particle could serve as the resonator [26, 27] while for the particle

size less than the wavelength laser resonators are formed by recurrent scattering [28]. In the present case, we speculate that the emission peak becomes narrower due to the preferential amplification at 591 nm within the FWHM. Ring cavities formed by recurrent scattering have different loss, and the lasing frequency is determined by the cavity resonance. Hence we propose that the granules in the reflector region, acting like a diffusive material, keep the light inside the system long enough for the amplification to become effective.

4 Conclusion

The appearance of a line in the molecular band spectrum in the visible region is quite striking. This indicates that the gain narrows at 591 nm. Recently, it is shown that in the diffraction pattern of the light of the firefly produced by a transmission grating the central principal diffraction maximum is predominantly yellow [12]. Since for a polychromatic source, the central principal diffraction maximum is of the same color as the source, we can now point towards this 591 nm emission as the reason for this. A similar reason could be cited for the double slit interference pattern presented in the same communication, where the yellow sector of the spectrum is again shown to be predominant. We propose that emission spectra of all the species of fireflies—more than 2000 in number, mostly in the tropics—be recorded again for the obvious reason of possibly obtaining the strong sharp line. One group of persons should take up this task for precisely assigning the peak wavelength as well as measuring the half-width of this line.

Acknowledgments This work is supported by Major Research Project F. No. 42-837/2013 (SR) of University Grants Commission, New Delhi, India.

References

1. Seliger, H.H., McElroy, W.D.: Quantum yield in oxidation of firefly luciferin. *Biochem. Biophys. Res. Commun.* **1**, 21–24 (1959)
2. Ando, Y., Niwa, K., Yamada, N., Enomoto, T., Irie, T., Kubota, H., Ohmiya, Y., Akiyama, H.: Firefly bioluminescence quantum yield and colour change by pH-sensitive green emission. *Nat. Photonics* **2**, 44–47 (2008)
3. Niwa, K., Ichino, Y., Ohmiya, Y.: Quantum yield measurements of firefly bioluminescence reactions using a commercial luminometer. *Chem. Lett.* **39**, 291–293 (2010)
4. Coblenz, W.W.: A physical study of the firefly. *Bull. Carneg. Instn.* **164**, 1–47 (1912)
5. Gohain Barua, A., Hazarika, S., Saikia, N.M., Baruah, G.D.: Bioluminescence emissions of the firefly *Luciola praeusta* Kiesenwetter 1874 (Coleoptera : Lampyridae : Luciolinae). *J. Biosci.* **34**, 287–292 (2009)
6. Viviani, V., Uchida, A., Suenaga, N., Ryufuku, M., Ohmiya, Y.: Thr226 is a key residue for bioluminescence spectra determination in beetle luciferases. *Biochem. Biophys. Res. Commun.* **280**, 1286–1291 (2001)
7. Biggley, W.H., Lloyd, J.E., Seliger, H.H.: The spectral distribution of firefly light II. *J. Gen. Physiol.* **50**, 1681–1692 (1967)
8. Bora, L., Baruah, G.D.: Bioluminescence emission of few species of fireflies. *Indian J. Phys.* **65B**, 551–557 (1991)
9. Iwasaka, M., Ueno, S.: Bioluminescence under static magnetic fields. *J. Appl. Phys.* **83**, 6456–6459 (1998)
10. Seliger, H.H., Buck, J.B., Fastie, W.G., McElroy, W.D.: The spectral distribution of firefly light. *J. Gen. Physiol.* **48**, 95–104 (1964)
11. Nakatsu, T., Ichiyama, S., Hiratake, J., Saldanha, A., Kobashi, N., Sakata, K., Kato, H.: Structural basis for the spectral difference in luciferase bioluminescence. *Nature* **440**, 372–376 (2006)

12. Dehingia, N., Baruah, D., Siam, C., Gohain Barua, A., Baruah, G.D.: Purkinje effect and bioluminescence of fireflies. *Curr. Sci.* **99**, 1425–1427 (2010)
13. Seliger, H.H., McElroy, W.D.: The colors of firefly bioluminescence: Enzyme configuration and species-specificity. *Proc. Natl. Acad. Sci. USA* **52**, 75–81 (1964)
14. Iwasaka, M., Miyashita, Y., Barua, A.G., Kurita, S., Owada, N.: Changes in the bioluminescence of firefly under pulsed and static magnetic fields. *J. Appl. Phys.* **109**, 07B303–1–3 (2011)
15. Cao, H., Zhao, Y.G., Ho, S.T., Seelig, E.W., Wang, Q.H., Chang, R.P.H.: Random laser action in semiconductor laser. *Phys. Rev. Lett.* **82**, 2278–2281 (1999)
16. Gohain Barua, A.: Modulations in the light of the firefly. *J. Biosci.* **38**, 9–12 (2013)
17. Gohain Barua, A., Rajbongshi, S.: The light of the firefly under the influence of ethyl acetate. *J. Biosci.* **35**, 183–186 (2010)
18. Peterson, M.K., Buck, J.: Light organ fine structure in certain asiatic fireflies. *Biol. Bull.* **135**, 335–348 (1968)
19. Ghiradella, H., Schmidt, J.T.: Fireflies at one hundred plus: a new look at flash control. *Integr. Comp. Biol.* **44**, 203–212 (2004)
20. Buck, J.B.: The anatomy and physiology of the light organ in fireflies. *Ann. N.Y. Acad. Sci.* **49**, 397–482 (1948)
21. Lund, E.J.: On the structure, physiology and use of photogenic organs, with special reference to the Lampyridae. *J. Exp. Zool.* **11**, 415–468 (1911)
22. Goh, K.S., Sheu, H.S., Hua, T.E., Kang, M.H., Li, C.W.: Uric acid spherulites in the reflector layer of firefly light organ. *PLoS ONE* **8**, e56406 (2013)
23. Gouedard, C., Husson, D., Sauteret, C., Auzel, F., Migus, A.: Generation of spatially incoherent short pulses in laser-pumped neodymium stoichiometric crystals and powders. *J. Opt. Soc. Am. B* **10**, 2358–2363 (1993)
24. Lawandy, N.M., Balachandran, R.M., Gomez, A.S.L., Sauvain, E.: Laser action in strongly scattering media. *Nature* **368**, 436–438 (1994)
25. Wiersma, D.S., Lagendijk, A.: Light diffusion with gain and random lasers. *Phys. Rev. E* **54**, 4256–4265 (1996)
26. Markushev, V.M., Zolin, V.F., Briskina, Ch. M.: Luminescence and stimulated emission of neodymium in sodium lanthanum molybdate powders. *Sov. J. Quantum Electron.* **16**, 281–283 (1986)
27. Ter-Gabrielyan, N.E., Markushev, V.M., Belan, V.R., Briskina, Ch. M., Zolin, V.F.: Stimulated emission spectra of powders of double sodium and lanthanum tetramolybdate. *Sov. J. Quantum Electron.* **21**, 32–33 (1991)
28. Wiersma, D.S., van Albada, M.P., van Tiggelen, B.A., Lagendijk, A.: Experimental evidence for recurrent multiple-scattering events of light in disordered media. *Phys. Rev. Lett.* **74**, 4193–4196 (1995)