The light of the firefly under the influence of ethyl acetate

ANURUP GOHAIN BARUA* and SUBHASH RAJBONGSHI

1Department of Physics, Gauhati University, Guwahati 781 014, India
2Department of Electronics and Communication Technology, Gauhati University, Guwahati 781 014, India

*Corresponding author (Email, agohainbarua@yahoo.com)

When a firefly is made to inhale ethyl acetate vapour, a constant glow appears after a few minutes from its abdominal lantern. This control experiment has been performed by a few workers to record the emission spectrum of the firefly. However, a time-resolved experiment performed by us on this continuous light emitted by the species *Luciola praeusta* Kiesenwetter 1874 (Coleoptera : Lampyridae : Luciolinae) reveals that it is composed of a continuous train of tiny pulses! The nature of the pulses suggests that an oscillatory chemical reaction continues in the microsecond time scale in the lantern of the anaesthetized firefly.

[Barua A G and Rajbongshi S 2010 The light of the firefly under the influence of ethyl acetate; J. Biosci. 35 183–186]

DOI 10.1007/s12038-010-0022-6

1. Introduction

Fireflies are among the most charismatic of all insects. Their spectacular flashing displays have inspired poets and scientists alike. The complicated chemical reactions involved in the production of firefly light have generated considerable interest among biochemists. The almost lossless chemiluminescence reaction has greatly attracted the attention of electro-optical physicists.

The generally accepted mechanism of firefly bioluminescence is a multi-step process occurring within photocytes of the abdominal lantern. In the first step, luciferase converts firefly D-luciferin into the corresponding enzyme-bound luciferyl adenylate. In the next step, luciferase amino acid residues are recruited to promote the addition of molecular oxygen to luciferin, which is then transferred to an electronic excited-state oxyluciferin molecule and carbon dioxide. In the final step, the rapid loss of energy of the excited state oxyluciferin molecule via a fluorescence pathway results in the emission of visible light.

There have been numerous investigations on the spectral distribution of bioluminescence. In some of the investigations, for example, those of Seliger et al. (1964), Biggley et al. (1967), Gohain Barua et al. (2009), the fireflies were forced to inhale ethyl acetate vapours, that is, they were anaesthetized. From the lantern of the insensible fireflies appeared a constant glow whose spectra were recorded. Till now, there has not been any effort to ‘see’ this continuous light in very small time scales. This report presents a significant finding of the light of the firefly under anaesthesia.

The flash communication system of fireflies involves precisely timed, rapid bursts of bioluminescence. The duration of a single flash has been reported to vary from about 70 ms (Branham and Greenfield 1996) to a few hundred milliseconds (Buck et al. 1963; Lloyd 1973; Barry et al. 1979; Saikia et al. 2001). It has recently been reported (Gohain Barua et al. 2009) that the flashes from the firefly species used in the present study consist of a large number of tiny pulses. About 30 000 pulses of a duration of approximately 2 μs form a flash that is about 100 ms in duration. On an average, the flashes are separated from one another by 800 ms, the minimum duration of separation being 150 ms.

2. Materials and methods

The firefly species *Luciola praeusta* Kiesenwetter 1874 (Coleoptera : Lampyridae : Luciolinae) which emits a constant glow in the lantern after ethyl acetate treatment is shown in figure 1. During early evening, a few specimens were collected from the campus of Gauhati University, India. One of them (the one flashing the brightest, of course!) was kept inside a test-tube, and a piece of cottonwool dipped
in ethyl acetate was placed in the mouth of that tube. The flashing was initially rapid; it slowed down gradually and, after 5–6 min, an apparently continuous glow appeared. The ethyl acetate-affected firefly was taken out of the test-tube and placed right on the window glass of the PHOTONIX XP 2050 photomultiplier tube (PMT) which was fixed in an upright position, i.e. with its window glass in the upward direction. This small adjustment was made to increase the signal-to-noise ratio. Care was taken to see that the firefly stayed in its normal sitting position: the lantern facing the PMT. Then the insect was covered by a chamber (described in Gohain Barua et al. 2009). The bias voltage applied to the PMT was 1.7 kv. The experimental arrangement along with the pre-amplifier circuit used for the time-resolved study is sketched in figure 2. Figure 3 shows the oscilloscope screen with the zero- or no-signal appearance, which indicates the noise level. The train of pulses within the continuous light for two specimens are shown in figures 4 and 5. A Sony Cyber-shot DSC-H7S camera was used to record these events from the oscilloscope screen. The experiments were conducted in the last week of October 2009 from about 18.00 h to about 22.00 h Indian Standard Time. The average temperature in the laboratory during the experiments was 28°C. Ten specimens of firefly were used in the experiment.

3. Results and discussion

It is evident from figures 4 and 5 that the light output is oscillatory. The existence of pulses within the apparent ‘dc’ light comes as a big surprise. The total duration of the pulses is about a couple of microseconds, in conformity with the duration observed earlier (Gohain Barua et al. 2009). The oscillatory nature of the pulses clearly points to an oscillatory reaction, such as the Belousov–Zhabotinsky (BZ) reaction (Belousov 1959; Zhabotinsky 1964), albeit on...
The light of the firefly under the influence of ethyl acetate

J. Biosci. 35(2), June 2010

The following similarities with the BZ reaction are discernible. The minimum-intensity pulse appearing about 1 μs from the left end of the screen could be termed as an ‘end state’. The end states are 2.5 μs in duration, separated from one another by 10 μs. In between two such states, there exist six pulses. The second cluster of three pulses appears to be the mirror image of the first cluster of three. Hence, the first cluster signifies movement of the reaction in one direction while the second cluster signifies that in the opposite direction. It is clear from the figure that the ‘threshold’ comes approximately 5 μs after the first end state, and the second end state comes approximately 5 μs after this threshold. The second pulses in both the clusters are the ones with the maximum intensity and a minimum duration of 1.5 μs. The numerical values presented here are remarkably constant in all the displays recorded by the oscilloscope. Though the anaesthetized firefly emits light non-stop without any flash, this signal is essentially a continuous train of pulses! This is certainly not a ‘dc’ signal.

Figure 4. (a) Appearance of the digital storage oscilloscope screen when a constant-glow firefly was placed on the borosilicate glass window of the photomultiplier tube, and (b) the smoothened pulses. One ‘end state’ is about 1 μs from the left end of the oscilloscope screen. One pulse after this state is the one with the maximum intensity. Then comes a relatively weaker pulse, and the process is repeated in the reverse direction till the other ‘end state’ is reached.

Figure 5. (a) Appearance of the digital storage oscilloscope screen when another constant-glow firefly was placed on the borosilicate glass window of the photomultiplier tube, and (b) the smoothened pulses.
Since the availability of oxygen is the immediate biochemical trigger for light production, we propose that the role of ethyl acetate is to inhibit the respiration of mitochondria, which densely pack the peripheral cytoplasm of photocytes (Trimmer et al. 2001), thereby ensuring a continuous oxygen supply to the luciferin-containing organelles (peroxisomes). It is worth mentioning here that mitochondria have been proposed to act as gatekeepers which control access of oxygen to the light-producing reactions in the more centrally located peroxisomes (Buck 1948; Ghiradella 1998). Thus, the oscillating chemical reaction goes on uninterrupted while the firefly is in an unconscious state. In other words, bursts of neural activity that stimulate the release of the primary neurotransmitter octopamine, which triggers the firefly lantern, continue unabated.

The possibility of the flash itself contributing to the off signal of the firefly lantern for fireflies in nitric oxide vapour has been suggested by Trimmer et al. (2001). The present finding automatically refutes this opinion. As the term flash means stoppage of the reaction for a few hundred milliseconds, basic questions such as ‘Why does the firefly stop emitting light transiently in the conscious state, while it emits non-stop in the unconscious state?’ need to be addressed again. In other words, there is scope for further study on the mechanism of the chemiluminescence reaction.

Acknowledgements

The firefly species used in the experiment was identified by Dr L A Ballantyne, School of Agricultural and Wine Sciences, Charles Sturt University, Australia. We also thank Professor Kalyanee Boruah for allowing us to use the facilities of the UHE Cosmic Ray Research Laboratory, and Mr Simanta Hazarika and Mr Pradip Deka for their help in the laboratory.

References

Belousov B P 1959 A periodic reaction and its mechanism; Compilation Abs. Radiation Med. 147 145
Buck J H 1948 The anatomy and physiology of the light organ in fireflies; Ann. N.Y. Acad. Sci. 49 397–482
Gohain Barua A, Hazarika S, Saikia N M and Baruah G D 2009 Bioluminescence emissions of the firefly Luciola praeusta Kiesenwetter 1874 (Coleoptera : Lampyridae : Luciolinae); J. Biosci. 34 287–292
Lloyd J E 1973 Fireflies of Melanesia: bioluminescence, mating behaviour, and synchronous flashing (Coleoptera: Lampyridae); Environ. Entomol. 2 991–1008
Seliger H H, Buck J B, Fastie W G and McElroy W D 1964 The spectral distribution of firefly light; J. Gen. Physiol. 48 95–104
Zhabotinsky A M 1964 Periodic processes of malonic acid oxidation in a liquid phase; Biofizika 9 306–311

MS received 9 December 2009; accepted 2 March 2010
ePublication: 8 April 2010

Corresponding editor: VIDYANAND NANJUNDIAH