Bioluminescence of fireflies and evaluation of firefly pulses in light of oscillatory chemical reactions*

J Saikia, R Changmai & G D Baruah

Laboratory of Non-linear Optics and Laser, Department of Physics, Dibrugarh University, Dibrugarh 786004

Received 24 July 2001; revised 8 October 2001; accepted 10 October 2001

The bioluminescence spectra of few species fireflies [species: Photinus pyralis, Coleoptera: Lampyridae; Phengodes lacticularis, Lampyridae and Photuris pennsylvanica, Lampyridae] have been photographed in the wavelength range 5000 - 6700 Å. The intensity distributions of the spectra have been investigated. The scanning of the individual firefly pulses have been recorded and it is shown that the intensity pattern of the pulses exhibit the oscillatory behaviour inside the metabolism of firefly. The nature of relaxation oscillation is also examined in the firefly emission.

1 Introduction

The emission of light by natural means is of considerable interest to biophysicists and biochemists due to the complicated reactions involved. Electro-optical physicists have noted an analogy between the in vivo emission of male fireflies and laser light. The spectral distribution of firefly light and the chemistry of firefly have been investigated earlier. Earlier it was a view that was generally accepted that the spectrum of a firefly is continuous in nature with a single peak. This view was also supported by the time resolved spectrometry. But the work of Baruah and coworkers indicate conclusively that distinct groups of bands do exist in the spectrum of fireflies. Observations on numerous specimens of fireflies have established the fact that the emitted light ranges in colour from green to yellow. But visual observations do not exactly indicate the nature of the bioluminescence spectrum. Fireflies do emit a significant percentage of colours in longer and as well as in the shorter wavelength side, which are not readily observable to the naked eye under usual conditions. Another important feature of the firefly emission is the oscillatory nature of its pulses. A detailed study of the pulses is expected to throw the much-needed light on the nature of the chemical reaction inside the metabolism of firefly. In the present work the authors describe the in vivo bioluminescence spectra of a large number of specimens belonging to three different species. The pulses originating form an individual specimen of firefly have been investigated.

2 Experimental Details

The bioluminescence spectra for three different species of fireflies (species A: Photinus pyralis, Coleoptera: Lampyridae, Species B: Phengodes lacticularis, Lampyridae and Species C: Photuris pennsylvanica, Lampyridae) were photographed on a glass spectrograph with a slit width of 100 μ. The experiments were conducted during early evening to midnight hours, local time, using fireflies collected just prior to the experiment. A single firefly was held immobile inside a cotton plug with its light organ positioned towards the slit. A glass spectrograph with a slit width of 100 μ. The experiments were conducted during early evening to midnight hours, local time, using fireflies collected just prior to the experiment. A single firefly was held immobile inside a cotton plug with its light organ positioned towards the slit. An exposure time for one and half hour was sufficient to record the spectra on ORWO Panchromatic film of speed 400 ASA. The intensity distributions of the spectra were measured on densitometer (AIMIL). Fig. 1 shows the spectra of the three main species of fireflies under investigation and Fig. 2 is the intensity profile of the spectra as measured on a densitometer. Some 50 common specimens have been selected in this way and their photographic records have been made. The firefly pulses for a longer interval of time can be investigated with the help of an experimental arrangement as described below. The specimen of firefly is kept immobilized by encapsulating it with a thin cotton-wool plug. The light-emitting organ is placed near the slit of a dark chamber. The light is

*Paper presented at NCOMF 2000; Indian School of Mines, Dhanbad 826 004
allowed to fall on a photomultiplier tube (Model, 1100, Indotherm Institute, Bombay) and is fed to a recorder (B-5000, Omniscribe recorder, Houston Instrument series). The pulses are recorded with the help of chart moving with 25 cm a minute. The record has been made for three different conditions, that is using a red filter, green filter and without using any filters. A part of the record is shown in Fig. 3. In Fig. 4 the oscillation pattern of a firefly pulses has been shown until it dies down naturally. The authors have recorded the oscillation patterns for 30 specimens.

3 Results and Discussion

As may be inferred from Fig. 3 the intensity of a pulse at first increases, reaches a maximum after three pulses and then decreases again. The pattern of intensity of the pulses in an average group of 10 pulses seem to be quite regular and indicates some sort of oscillatory behaviour in chemical reaction inside the metabolism. It is worthwhile to note that chemical reactions can oscillate spontaneously and during the last 20 years significant works have been carried out on such experimental systems known as chemical oscillators.\textsuperscript{9-15} As may be inferred form Fig. 4, there is an increase in intensity of the second oscillating group (consisting of about 10 pulses) and after that the oscillations gradually die down indicating some sort of damping being introduced in the experimental system.

![Intensity vs. Wavelength](image)

Fig. 2 — Densitometer tracing of the bioluminescence spectra

![Time Resolved Pulses](image)

Fig. 3 — Time resolved pulses of a firefly: (a) without filter, (b) with green filter and (c) with red filter

![Bioluminescence Spectra](image)

Fig. 1 — Bioluminescence spectra of three species of fireflies photographs on a glass spectrograph
Fig. 4 — A typical time resolved pulses of a firefly indicating the oscillating nature.

The nature of the pulses has been observed in almost all the firefly specimens being investigated in the work. The considerations set forth indicate that one of the most striking characteristics of firefly emission is its oscillatory nature. The oscillating chemical reactions\(^1\) and the so-called BZ reactions, as it came to be called, is considered as a good model for complex systems. The mechanisms of chemical oscillations can be very complex. The BZ reaction itself is thought to involve more than 20 elementary steps but as many of them came to equilibrium rapidly, these allow the kinetic to be reduced to few steps only. The emission of firefly is recognized as due to a chemical reaction which leads to a release of energy due to an oxidation process. The enzymes that produce light are called luciferase and the substrate luciferins. It is also believed that the firefly flash is triggered off by a nerve impulse delivered to the luminescence gland. The reaction is singular in that one quantum of light is produced for each molecule of luciferin oxidized. The mechanism of chemical oscillations in firefly is yet to be fully established. But it is reasonable to believe that reactions similar to BZ reactions exist inside the firefly metabolism.

As shown in Figs 3 and 4 the phenomenon of relaxation oscillation is also strikingly demonstrated in the bioluminescence pulses of fireflies. One of the characteristic features of the pulse is that the growth of intensity is very fast as compared to the decay. Fig. 5 indicates a pulse fitted with the help of a computer. The type of decay curve shown here may be conveniently represented by an equation of the type \( I = I_0 \exp (-\gamma t) \), where \( I_0 \) is the intensity in its maximum position and \( \gamma \) is the decay constant. The salient feature of all the firefly pulse is that the intensity always grows from a non-zero value. In contrast to the decay curve the growth is very fast. Earlier workers have estimated this growth to be in the order of microseconds\(^2\). The growth and decay of a typical firefly pulse is in fact analogous to the
phenomena like the charge and discharge of a condenser, firing of a rocket during its initial period or a neuron pulse.

The evaluation of the in vivo bioluminescence spectra of the species of fireflies under consideration shows some general agreement with earlier in vivo measurements, but indicate few disagreements. The asymmetric nature of the intensity profile is found to be present in all the measurements. But the peak wavelengths of the spectra were determined at different values by different workers. The differences may be attributed to the differences in measurement technique. Table 1 shows the spectral range of three different species of fireflies.

From Table 1 and also from Fig. 1 it is apparent that the spectra of firefly consists of two distinct maxima or bands. This is contrary to the earlier belief that firefly spectra are continuous and do not possess any discrete structure. The long wavelength system of band is weak as compared to the short wavelength system. At this stage it is also difficult to identify the origin of the discrete structure in the spectra or to make any correlation with the oscillatory chemical reaction. However, a proper interpretation of the spectra will throw the much-needed light on the nature of the oscillatory reaction itself.

4 Conclusion

In the present study, the authors have used the spectroscopic techniques to analyze the bioluminescence emission of fireflies. The spectra presumably show discrete structure and it may be considered as important from the point of view of cell-biology. In the present work, the time-resolved scanning of firefly pulses has been considered as the manifestation of chemical oscillators. It must be noted that there is an inherent instability of light processes. The authors are dealing with a variety of steady states, each of which may be transitory and soon shift to a new steady state; indeed, oscillations in the concentration of intracellular chemical species may occur. There is a truly remarkable range of speed of biochemical reactions varying from diffusion-controlled reactions to the light activated primary reactions of photosynthesis in the picosecond time range. Finally, there is a complexity of the chemical pathways, many of which remain to be elucidated.

Acknowledgement

The authors are grateful to Prof R K Garvia of the Department of Physics, Manipur University, Manipur, for allowing use of the facilities in connection with the scanning of the firefly light pulses.

References