QUANTUM YIELD IN THE OXIDATION OF FIREFLY LUCIFERIN

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In bioluminescence the energy required for the production of light is obtained by the enzymatic oxidation of a specific substrate, luciferin. In the firefly, Photinus pyralis, oxidation of luciferyl-adenylate generates an excited state which subsequently returns to the ground state by the emission of visible light. The details relating to the in vitro function of ATP, luciferyl- and oxyluciferyl adenylate in firefly light emission have been presented previously (Rhodes and McElroy, 1958). The detailed mechanism of the oxidative reaction and of the subsequent generation of an excited state capable of emitting light with the equivalent of over 50 kcal per mole remains obscure. Essential information for differentiating between various proposed mechanisms has been difficult to obtain because of certain features of the enzyme catalyzed reaction. In considering these various mechanisms it became essential to know the number of oxygen and luciferin molecules used for each light quantum emitted. Recently we have measured the absolute number of light quanta emitted per luciferin molecule oxidized. This ratio we define as the quantum yield Q.

Purified crystalline luciferin and luciferase were used in the measurements of the quantum yield and in the subsequent spectral emission measurements. Luciferin concentrations were determined spectrophotometrically, based on a molar extinction coefficient of 18,800 at 327 mμ at a pH of 7.6. In the actual experiments the total luciferin was varied from 10^{-11} mole to 5×10^{-10} mole. The geometry consisted of a cylindrical

quartz cell 1 inch in diameter with a flat bottom optically coupled to the flat face of a DuMont 6292 phototube. Two ml of 0.025M glycyl-glycine (pH 7.6), 0.1 ml of 0.1 M magnesium sulfate, 0.1 ml of enzyme solution (approximately 10 x the molarity of the luciferin solution) and 0.1 ml of luciferin solution were added to the cell and stirred. Then approximately 10^{-6} mole of ATP in aqueous solution was injected into the cell with a hypodermic syringe. The cell and phototube were covered with a light-tight magnetic shield and the high voltage switched on to the phototube. The total charge collected at the anode of the phototube was measured with a D.C. amplifier and a condenser integrating circuit. The sensitivity was adjusted so that 10^{-5} coulombs activated a sensitive relay which discharged the condenser; the total charge collected could then be determined by counting the number of these discharges on a recorder chart. In this way the rate of light emission as well as the integral of the light emission could be observed. Details concerning the absolute calibration of the experimental arrangement will be published elsewhere.

Bioluminescence emission spectra were measured in an F/3 grating monochromator with a dispersion of 2μ per millimeter. The detector was an RCA 7326 phototube sensitive out to 800 mμ. The spectrum of firefly light in glycyl-glycine buffer at pH 7.6 consists of a single emission band with a peak at 562 mμ.

A frequency distribution for 39 independent measurements of Q is shown in figure 1. The average value for all these measurements is 0.88 ± 0.25 demonstrating that one light quantum is emitted for every luciferin molecule oxidized. This luminescence efficiency is at least twenty fold greater than has been observed previously for other bioluminescent reactions (Eymers and Van Schouwenberg 1937, Cormier and Totter 1957). It is significant that Q is not influenced by temperature over the range of 3 to 25°C. The emission of light from singlet excited states of

aromatic molecules usually takes place with a mean life time of 10^{-9} - 10^{-8} seconds. During this time the molecules can make approximately 10^{3} - 10^{4} quenching collisions. The absence of a temperature effect on the quantum yield and the fact that Q is unity, indicate that the excited state does not readily interact with molecules in the environment. It has been shown previously that oxyluciferyl-adenylate is tightly bound to the enzyme molecule and it seems likely that this tight complex is important in restricting the reactivity of the excited

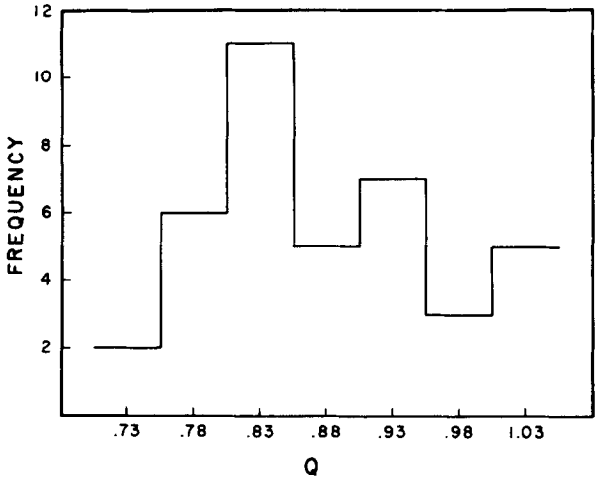

Fig. 1 Frequency distribution of 39 independent measurements of Q.

state. It is, perhaps, significant that the fluorescence of the luciferase overlaps the broad absorption band of both luciferin and oxyluciferin at neutral pH. Possibly the energy released in the oxidative process is retained more readily in the enzyme-substrate complex from which it can be trapped at an emitting site. When light emission is measured at pH values below 7.0 the quantum yield decreases markedly. These results would also suggest that the protein is important in the transfer of energy required for light emission or, at least, in the isolation of the activated molecule from the environment.

Acknowledgments: the authors would like to thank Mrs. Linda Geiger for her valuable technical assistance and Dr. William Eastie for

the design and construction of the F/3 spectrometer. This research was sponsored in part by a grant from USAEC Division of Biology and Medicine, the National Science Foundation, and one of us (HHS) would like to express his thanks to the John Simon Guggenheim Memorial Foundation for a Fellowship during the past year.

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Received June 27, 1959