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Studies on the Luminescence Reaction of a Myctophid Fish, *Diaphus elucens* BRAUER

F. I. TSUJI* and Y. HANEDA**

(With 1 Text-figure and 1 Table)

スイトウハダカ *Diaphus elucens* の発光反応

フレデリック辻一郎　羽根田弥太

生物の発光反応において Luciferin Luciferase 反応は同一種生物の間か，近縁な生物の間にのみ，みられるのは常識であった。ところが発光魚キンメモドキ *Parapriacanthus ransonneti* と甲殻類のウミボタル *Cypridina hilgendorfii* との間に強い交叉反応があることが証明せられ ((HANEDA, JOHNSON 1958)，ツマグロイシモチ *Apogon ellioti* (HANEDA, JOHNSON and SIE)，フィリッピンの発光イシモチ類 *Apogon, Arcamia, Rhabdamia* (HANEDA, TSUJI and SUGIYAMA 1969) とウミボタル，および魚の相互の間にも交叉反応があることが明らかになった。しかし，反応は，魚の発光器の構造，生化学的研究，食餌などの研究から，これらの魚は摂取した発光動物のルシフェリンを消化管を通して，発光器に送っていると推察されるようになって，魚とウミボタルとの間の交叉反応は結局はウミボタルの反応であり，当然であると考えられるに至った。1967 年メキシコ湾の発光魚 *Porichthys prosissimus* とウミボタルとの間に交叉反応があることが証明せられ (CORMIER CRANE and NAKANO 1967)，さらにカリフォルニア沿岸の発光魚 *Porichthys notatus* とウミボタルの間にも発光交叉反応のあることが確められた (TSUJI, HANEAD, LYNCH, and SUGIYAMA in Press)。

これらの発光魚の発光器はキンメモドキや，Apogon のそれのように，消化管との連絡がなく，皮膚発光器となっている。これらの魚もウミボタルか他の発光甲殻類を食べて，その摂取動物のルシフェリンを利用していると考えれば，ルシフェリンは循環系を通って発光器に到達するが，分子量の大きいルシフェラーゼは魚自身が作るものであろうか，あるいはまた，海棲動物の間にはウミボタルと共通のルシフェリンが広く他の発光生物まで分布しているものであろうか，現在，未解決のまま残されている。

スイトウハダカ *Diaphus elucens* はハダカイワシ科 Myctophidae の中でも，とくに頭部の発光器の大きな種類である。材料は駿河湾，由比沖のサクラエビ網に混入したもので，新鮮な材料を冷凍し，冷凍乾燥した。

本魚の頭部発光器，体側発光器，筋肉，胃の内容，幽門垂，肝臓と，ウミボタル *Cypridina hilgendorfii* との間の Luciferin, Lueiferase 交叉反応を調べたところ，頭部発光器，体側発光器では明らかに強く発光し，筋肉，胃内容が弱く発光し，発光反応は陽性であつたが，幽門垂，肝臓は陰性であつた。

なお，これらの発光器，筋肉等といくつかの Cofactor が発光を誘発するか，どうかを調べた，1% hydrogen peroxide, crystaline beef liver catalase では反応があり，nicotinamide adenosine dinucleotid, NADH, HADP, NADPH, ATP. Coenzyme A では反応はなかった。

これらの反応は他のハダカイワシ，例えば南カリフォルニア沿岸の *Stenobrachius leucopsarus* の皮膚発光器，尾鰭基部の発光板でも同様の反応を認めた。

従って，ハダカイワシの 2 種の発光機構はウミボタルの発光機構と非常ににたものと考えられる。

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* Department of Biophysics and Microbiology, University of Pittsburgh, and Veterans Hospital, Pittsburgh, Pennsylvania. U.S.A,

** Yokosuka City Museum, Yokosuka, Japan.

Among the most common and widely distributed deep-sea fishes with photophores are the myctophids or lantern fishes of the family Myctophidae. Their photophores are arranged in species-specific patterns over the ventral and lateral surfaces of the body. The photophores consist of small dermal structures containing luminous tissue, lens, and reflector. Some species possess, in addition, a large nasal organ, while others have prominent organs on the caudal peduncle. The photophores of a freshly collected myctophid emit a blue light with an emission maximum of around 470 mμ[1], but the light usually disappears irreversibly in about 10 min or less. Little is known about the bioluminescence mechanism.

Anctil and Gruchy[2] have shown that the dermal photophores of the myctophids *Tarletonbeania crenularis taylori* and *Protomyctophum thompsoni* luminesce when hydrogen peroxide is injected into or applied to the body, but not the photophores of the myctophid *Stenobrachius leucopsarus*, suggesting a peroxidase mechamism for the bioluminescence reaction in the two fish. Mitchell[3] has reported weak light emissions from cell-free extracts of myctophid photophores prepared in Tris (hydroxymethyl) aminomethane (Tris)-ethylenediamine tetraacetate (EDTA) solution, but luminescence of such extracts did not respond to stimulation by reduced nicotinamide-adenine dinucleotide (NADH), riboflavin monomucleotide (FMN), reduced riboflavin mononucleotide ($FMNH_2$), adenosine triphosphate (ATP), sodium thiosulfate, excess calcium ions, and excess hydrogen ions. We wish to report that photophore extracts of the myctophid, *Diaphus elucens* Brauer, cross-react to give light with purified luciferin (substrate) and luciferase (enzyme) of the luminous marine ostracod crustacean, *Cyridina hilgendorfii*.

Specimens of *D. elucens* were collected at night with nets off Yui, Suruga Bay, Japan, at depths of 20–30 m as they migrated toward the surface from areas of the Bay 1,000–1,600 m deep. They were frozen in dry-ice and then stored up to 3 months at −35°C until used. Specimens measured 102–140 mm in length from tip of snout to tail base and 24–31 mm in body depth. The distribution

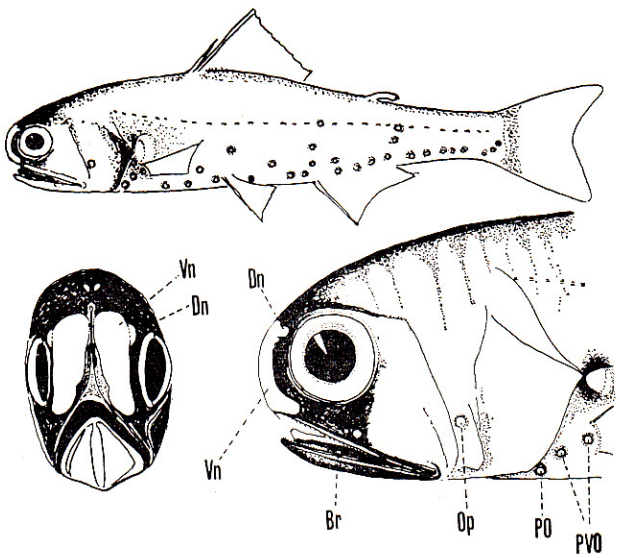

Fig. 1. *Diaphus elucens*, showing luminous organs on trunk and head. Note the large ventro-nasal organ (Vn) and the dorso-nasal organ (Dn). Other luminous organs: branchiostegal (Br), opercular (Op), pectoral (PO), and subpectoral (PVO). Drawn from actual specimen. Terminology of the photophores is according to Bolin.[4]

of the luminous organs is shown in Fig. 1. The large nasal organs (Vn, Dn) on both sides of the snout and dermal photophores were removed with a pair of ophthalmic surgical scissors and each used for the biochemical studies. Muscle tissue was excised from deep within the flank, after removing the skin and underlying muscle and rinsing with distilled water. The three different tissues were weighed and ground separately in 6 ml. of 0.05 M Tris (pH 8.0) in an all-glass homogenizer chilled in an ice bath. The homogenate was centrifuged at 12,500 g for 10 min at 4°C. Extracts of individual stomach, pyloric caecum, and liver were also prepared in a similar manner from well-rinsed tissue. All extracts used were dark. For the luciferin-luciferase cross-reaction tests, two kinds of reaction mixtures were studied. The first consisted of 1 ml. of extract, 0.2 ml. of *Cypridina* luciferin, and 2 ml. of 0.2 M Tris (pH 8.0). The second consisted of 1ml. of extract, 1 ml of *Cypridina* luciferase, and 1.2 ml. of 0.2 M Tris (pH 8.0). The extract in each case was injected with a syringe into a reaction cell containing the remainder of the reaction mixture. Tris (pH 8.0) substituted for extract in the controls. Since the *Cypridina* luminescence reaction (λ_{max}=465 mm) involves the oxidation of luciferin (which has a complex structure containing tryptamine, isoleucine, and arginine moieties) by molecular oxygen catalyzed by luciferase[5], the injection of either luciferin or luciferase into a solution containing the other component would initiate the light reaction. Highly purified *Cypridina* luciferin (in 0.1 N HCl) and luciferase (1.73×10^{-3} mg/ml., in water) wre used[6,7]. The luciferin solution was stored under argon in an ice bath during use to prevent loss of activity from autoxidation. All light intensities were measured in arbitrary light units with a MacNichol type photomultiplier (RCA 1P21) photometer[8].

Based on initial light intensities, extracts of nasal and dermal photophores were the most active when cross-reacted with *Cypridina* luciferin, followed by muscle. In a representative specimen the wet weights of the homogenized tissues were as follows: nasal organ, 0.20 g; dermal photophores (24), 0.45 g; and muscle, 0.80 g. The corresponding initial light intensities were 15, 28, and 7. The light intensities produced by the extracts of nasal organ and dermal photophores, however, varied from specimen to specimen and were often 3–5 times higher in one than in another. Stomach extracts gave about the same light intensity as muscle. When cross-reacted with *Cypridina* luciferase, the above four extracts produced substantially less light than with *Cypridina* luciferin, and in most cases the intensities were insignificant. A few extracts of nasal organ and dermal photophores, however, emitted as bright light with *Cypridina* luciferase as with *Cypridina* luciferin. Such extracts did not emit light when cross-reacted with extracts that were active with *Cypridina* luciferin. Some of these extracts (highly active with *Cypridina* luciferase) emitted light when injected into 0.05 M Tris (pH 8.0). Extracts of pyloric caecum and liver did not produce light with either *Cypridina* luciferin or luciferase.

Tests for stimulatory activity by various cofactors were carried out with 1 mg of cofactor freshly dissolved in 1 ml. of 0.2 M Tris (pH 8.0). Each cofactor was injected into an active extract or into 3.2 ml. of the above reaction mixture containing either *Cypridina* luciferin or luciferase. One ml. of 1% hydrogen peroxide and 1 ml. of crystalline beef liver catalase (Worthington) (1 mg/ml., in water) were injected into 2 ml. of active extract. Negative results were obtained with the following cofactors: nicotinamide adenine dinucleotide (NAD^+), NADH, nicotinamide-adenine dinucleotide phosphate ($NADP^+$), reduced nicotinamide-adenine dinucleotide phosphate (NADPH), ATP, and coenzyme A. A slight stimulation of light emission was obtained with hydrogen peroxide, and with

hydrogen peroxide mixed with catalase, but the intensity was less than 1% of the intensity obtained with *Cypridina* luciferin. Catalase alone produced no light.

The extract used in the kinetic measurements was prepared by homogenizing 100 dermal photophores in 10 ml. of 0.2 M Tris (pH 8.0), as described above. The extract was further dialyzed for approximately 24 h at 4°C against 4 changes of distilled water, centrifuged, and diluted with an equal volume of 0.2 M Tris (pH 8.0). It was then mixed with *Cypridina* luciferin and 0.2 M Tris (pH 8.0) to obtain the decay curves. Fig. 2 shows the light intensities (which are directly proportional to reaction rates) plotted against reaction time according to the equation for a first order reaction. The calculated rate constants were for curve *a*, $4.59\times10^{-2}\text{sec}^{-1}$; curve *b*, $2.15\times10^{-2}\text{sec}^{-1}$; and curve *c*, $1.99\times10^{-2}\text{sec}^{-1}$. The rate constants are seen to be very nearly proportional to extract concentration (curves *a*, *b*) and independent of luciferin concentration (curves *b*, *c*). The luminescence reaction, therefore, is first order, and resembles the *Cypridina* reaction[6]. The reason for the high initial rate is unknown, but it also occurs in the *Cypridina* reaction[6]. When undialyzed extract was used, the decay curves showed a marked curvature from the beginning, except at extreme extract dilution. Dialysis produced a 40–50 fold increase in extract activity and caused the decay curve to become linear, possibly by removing an inhibitor. With longer dialysis, however, a precipitate formed which contained nearly all of the activity. When an homogenate of the precipitate in 0.2 M Tris (pH 8.0) was centrifuged, most of the activity appeared in the supernatnat.

In the immunological studies, the extract was reacted with immunoglobulin-G (IgG) isolated from the antisera of rabbits immunized against purified *Cypridina* luciferase[6]. Three different concentrations of IgG were used (1×10^{-2}, 3×10^{-2}, and 9×10^{-2} mg/ml., in 0.2 M Tris (pH 8.0)). The reaction mixture consisted of 1 ml. IgG and 1 ml. of either dialyzed extract or 1 ml. of *Cypridina* luciferase (4.32×10^{-7} mg/ml., diluted from the above water solution with 0.2 M Tris (pH 8.0)). Controls consisted of 0.2 M Tris (pH 8.0) in place of IgG. After 1 h incubation at 4°C, 0.2 ml. of *Cypridina* luciferin diluted with 1 ml. of 0.2 M Tris (pH 8.0) was injected into the mixture. The observed initial light intensities are given in Table 1. The data show clearly that activities of the extract and *Cypridina* luciferase are inhibited by IgG, but that *Cypridina* luciferase is inhibited to a greater extent than the extract. Since *Cypridina* luciferase is inhibited by antisera of rabbits immunized against *Cypridina* luciferase, but not by normal sera[10], the results suggest that the extract contains an enzyme similar to *Cypridina* luciferase.

The requirement for oxygen was tested by placing 5 ml. of dialyzed extract+3 ml. of 0.2 M Tris (pH 8.0) in one arm, and 1 ml. of *Cypridina* luciferin+7 ml. of 0.2 M Tris (pH 8.0) in a second arm, of an all-glass, mixing apparatus. The two solutions in the arms were bubbled with 99.99% argon for 15 min and the apparatus was evacuated for 2 min with a vacuum pump. The arm containing the *Cypridina* luciferin was placed before the window of the photomultiplier and the extract was added under vacuum. No light was produced. After 30 sec, air was admitted into the mixture through a small capillary. A burst of light occurred and the luminescence then decayed according to the first order equation. The intensity was very nearly the same as that of a control mixed in air. A similar result was also obtained when 5 ml. of *Cypridina* luciferase (4.32×10^{-7} mg/ml., diluted from the water solution with 0.2 M Tris (pH 8.0))+3 ml. of 0.2 M Tris (pH 8.0) were substituted in the first arm.

The above light-emitting cross-reactions of *Cypridina* luciferin and luciferase have recently been

Table 1. Effect of Rabbit IgG on *Diaphus* Photophore Extract and *Cypridina* Luciferase

Mixture	Initial Light Intensity
Diaphus extract, control	42
Diaphus extract $+1\times10^{-2}$ mg IgG	27
Diaphus extract $+3\times10^{-2}$ mg IgG	21
Diaphus extract $+9\times10^{-2}$ mg IgG	14
Cypridina luciferase, control	42
Cypridina luciferase $+1\times10^{-2}$ mg IgG	23
Cypridina luciferase $+3\times10^{-2}$ mg IgG	15
Cypridina luciferase $+9\times10^{-2}$ mg IgG	10

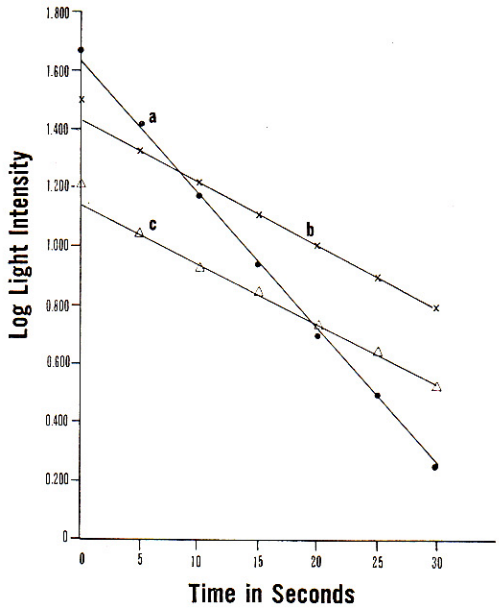

Fig. 2. Luminescence decay curves of dialyzed *Diaphus* photophore extract-*Cypridina* luciferin mixtures. Curve *a*, 1 ml. extract, 0.2 ml. luciferin, 2 ml. of 0.2 M Tris (pH 8.0); curve *b*, 0.5 ml. extract, 0.2 ml. luciferin, 2.5 ml. of 0.2 M Tris (pH 8.0); and curve *c*, 0.5 ml. extract, 0.1 ml. luciferin, 2.6 ml. of 0.2 M Tris (pH 8.0).

confirmed with light organ extracts of another myctophid, *Stenobrachius leucopsarus*, collected off the coast of southern California. Similar results were obtained with extracts of the dermal photophores, and supracaudal and infracaudal organs, prepared and tested as described above. It should be noted that members of the genus *Diaphus* lack caudal organs, whereas members of the genus *Stenobrachius* lack a large nasal organ. We conclude that in at least two species of myctophids, the biochemical mechanism for luminescence involves a luciferin and luciferase very similar to the luciferin and luciferase of *Cypridina*.

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