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Biochemistry of Bioluminescence in the Deep-sea Squid, *Watasenia scintillans*

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The deep-sea squid, *Watasenia scintillans*, emits a bright blue luminescence that is readily visible to the dark-adapted eye. The light originates from numerous, tiny photophores located on the ventral side of the body, arms, and tentacles. The light emission ceases on the death of the animal. Previous studies have shown that cell-free extracts of the animal do not give the classical luciferin (substrate) -luciferase (enzyme) reaction. However, recently it was shown that a homogenate prepared from the arm photophores emits a bright, long-lasting light when mixed with a solution of adenosine 5'-triphosphate (ATP) . The light emission is due to the oxidation of luciferin by molecular oxygen, catalyzed by luciferase. The luciferin is the disulfate of coelenterazine, a compound used as substrate in the bioluminescence reactions of many marine organisms. The role of ATP is still unknown, but it may be involved in the transfer of sulfate groups to the hydroxyl groups of coelenterazine to form luciferin. The luciferase is strongly bound to membrane and cannot be easily solubilized. Thus, the enzyme is not present in a cell (membrane) -free extract, but the membrane, when added to an *in vitro* bioluminescence reaction, is fully active as the luciferase.

Key words : ATP, coelenterazine disulfate, luciferase, luciferin, membrane.

Bioluminescence is both a biochemical and physical process in which light is emitted by living organisms. The ability to produce light is widely distributed in nature and is possessed by organisms that are both terrestrial and marine. The firefly is the best known example, but there are numerous other organisms, especially in the marine environment that are luminescent. They include the bacteria, dinoflagellates, jellyfishes, sea pens, polychaetes, tunicates, crustaceans, cephalopods and fish. The fish is the highest form of life known

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$$\text{Luciferin} + O_2 \xrightarrow{\text{Luciferase}} \text{Oxidized products} + \text{Light}$$

Fig.1 General scheme for bioluminescence reaction in marine and terrestrial organisms.

to luminesce. In some higher animals, such as the squid and fish, the light is produced in complex morphological structures called "photophores," which contain specialized cells called "photocytes." These cells emit the light. In addition, a photophore usually has a reflector surrounding the photocyte to collect the emitted light and direct it into the lens for transmission to the outside. The photophore is also innervated by nerves that regulate the intensity and duration of the light emission by means of a shutter or chromatophores. Some photophores also possess a color filter to modify the color of the light.

The light-emitting reaction takes place in the photocyte and is due to an enzyme-substrate (chemical) reaction in which luciferin (substrate) is oxidized by molecular oxygen, catalyzed by luciferase (enzyme) (Fig.1) . In bioluminescence reactions very close to 100% of the energy of the reaction is emitted as light so that no energy is converted to heat. This is why a luminescing firefly, held in the hand, does not give off the slightest amount of heat-all of the chemical energy is emitted in the form of light. We, therefore, call this "cold light." There is another way in which light originates from photophores and this will be mentioned shortly.

In this paper, we describe what is known about the bioluminescence in the small, deep-sea squid, *Watasenia scintillans* (Fig.2) . This squid appears in Toyama Bay, Japan, every year in enormous numbers in the spring. They come inshore to breed and the incoming population consists predominantly (90%) of females carrying fertilized eggs; the fate of the males is unknown but presumably they have died earlier. *Watasenia* measures about 6cm in mantle length and has some 800 photophores distributed on its ventral side. The most prominent photophores are found on the tip of the fourth pair of arms. On each arm there are three minute organs colored black and these emit bright flashes of light when the squid is mechanically disturbed. The arm photophores were first described by Watase (1905) and the ultrastructure has been studies by Okada (1966) . Because of the flashing behavior of *Watasenia*, the squid is named "hotaru ika" or "firefly squid" by the Japanese.

Luminescent squids produce light either by harboring symbiotic luminous bacteria in their photophores or by possessing their own light-emitting compounds for use in an endogenous reaction. In the case of squids that produce light by means of symbiotic luminous bacteria, the photophores contain, instead

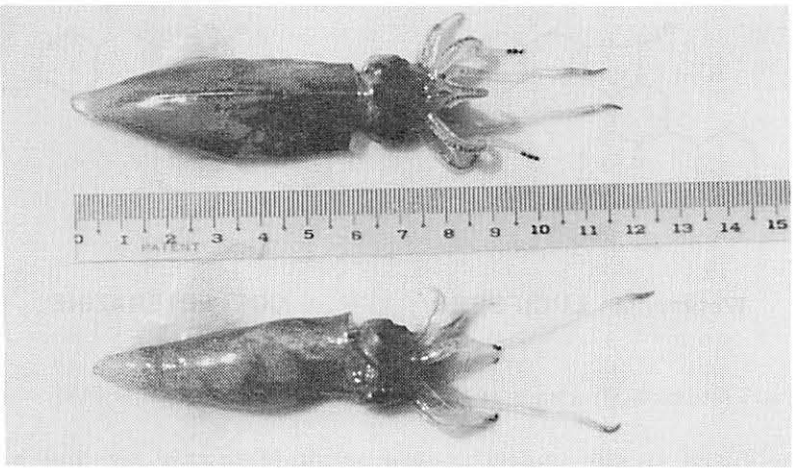
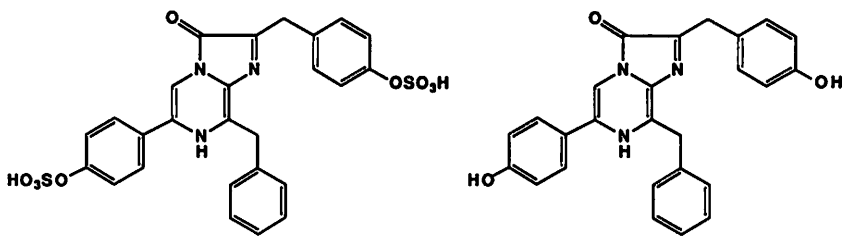


Fig.2 Photograph of specimens of *Watasenia scintillans* showing three tiny black photophores at the tip of each arm.

of photocytes, specialized ducts in which countless numbers of symbiotic luminous bacteria are present. The squid provides nutrients to the bacteria and the bacteria contribute a source of light. A similar arrangement exists among many luminescent fish.

Almost all that we know about the endogenous type of bioluminescence reaction in squids have come from studies on the arm organs of *W. scintillans*. One problem with *Watasenia* is that on the death of the animal, the photophores lose all activity, becoming dark. All attempts, until recently, to obtain an active, cell-free extract of the arm organs have resulted in failure. In order to overcome this problem, Goto and his associates (Goto, et al., 1974; Inoue, et al., 1975, 1976, 1977) examined the products present in pooled extracts of the arm organs of more than 10,000 specimens of *Watasenia*. From an analysis of the products, consisting mainly of oxyluciferin, they were able to determine the structure of oxyluciferin and deduce the structure of luciferin. It was found that *Watasenia* luciferin is a disulfate of coelenterazine (Fig.3). Further studies by Goto and his associates showed that the liver contained relatively large amounts of coelenterazine and little of coelenterazine disulfate, whereas the photophores contained relatively large amounts of the disulfate of coelenterazine but only limited amounts of coelenterazine. They therefore concluded that the liver served as a storage organ for the coelenterazine and that the squid converted the coelenterazine to the disulfate form for use in the photophores.



Watasenia LUCIFERIN COELENTERAZINE

Fig.3 Chemical structures of *Watasenia* luciferin and coelenterazine.

Further progress in the understanding of the *Watasenia* reaction have come from the studies of Tsuji (1985) . He discovered that light emission from a dark, cold-water extract of the photophores was stimulated markedly in light intensity by the addition of adenosine 5'-triphosphate (ATP) , but not by the other nucleoside 5'-triphosphates (guanosine 5'-triphosphate, cytidine 5'-triphosphate and uridine 5'-triphosphate) . However, it is not known how ATP acts in the light-emitting reaction. It might be expected that ATP would serve as a means of transferring a sulfate group to each of the -OH groups in coelenterazine, such as by an adenyl sulfate intermediate, but no proof has been obtained for its involvement in such a reaction. It is still possible that ATP may be involved in such a transfer by an entirely new mechanism.

Attempts have also been made to purify the enzyme, luciferase, from the arm organs of *Watasenia*. Inoue et al. (1975) reported that they were unable to prepare an active, cell-free extract of the photophores. Such a soluble extract is a prerequisite for studying the reaction *in vitro* in order to identify all of the components of the reaction. Tsuji (1985) found that the luciferase is membrane-bound and centrifuging an extract of the photophores caused the luciferase to be sedimented in the pellet. The pellet could be resuspended in buffer and mixed with the supernatant of the extract to regain activity on adding ATP. The luciferase is apparently so strongly bound to membrane that it cannot be solubilized even using various detergents. The substrate, coelenterazine disulfate, on the other hand, is readily soluble in aqueous solution. Thus, an active, light-emitting mixture can be prepared by mixing the supernatant of a photophore extract with the pellet. Injection of a solution of ATP to such a mixture results in a long-lasting luminescence, identical to the luminescence of the photophores (Tsuji, unpublished results) . In summary, the above results indicate that the components of the *Watasenia* reaction consists of luciferin (coelenterazine disulfate present in supernatant) , luciferase (bound to mem-

brane in pellet) , ATP and molecular oxygen. The *Watasenia* reaction therefore would classified as a bioluminescence reaction in which coelenterazine disulfate is oxidized by molecular oxygen, catalyzed by luciferase, yielding light and products.

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深海性ホタルイカにおける生物発光の生化学

フレデリック・I・辻

深海性イカの1種ホタルイカは暗順応した肉眼で容易に見ることができる青く輝いた光を発する。この光は胴体の腹側，腕及び触腕にある多数の小さな発光器から出される。発光は動物体が死ぬと止む。これまでの研究により，この動物体の無細胞抽出物は古典的なルシフェリン（基質）－ルシフェラーゼ（酵素）反応を示さないことがわかっている。しかし，最近になって腕の発光器のホモジェネートはアデノシン5’－三燐酸（ＡＴＰ）溶液と混合すると，輝いた，長時間持続する光を発することが明らかにされた。発光はルシフェラーゼの働きによって，ルシフェリンが分子状酸素で酸化されることによって起こる。このルシフェリンはケレンテラジンの二硫酸塩で，多くの海洋生物の生物発光反応の基質として用いられている化合物である。ＡＴＰの役割についてはまだ明らかにされていないが，ルシフェリン形成に際して硫酸基をケランテラジンの水酸基へ転移させるのに関与しているのかもしれない。ルシフェラーゼは強く膜に結合しており，容易には溶出しない。このため，この酵素は無細胞（膜）抽出物には存在しない。しかし，膜は*in vitro*の生物発光反応に加えるとルシフェラーゼとして十分な活性を示す。