

## Bioluminescence of the terrestrial snail *Quantula striata*: chemical nature of the luminescence system<sup>1</sup>

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発光カタツムリ *Quantula striata* の発光系の化学的性質<sup>1</sup>

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シンガポールの発光カタツムリ *Quantula striata* は人工的な刺激によって発光せず、また一度凍結したあとは発光させることができない。しかし冷凍試料の抽出液は、 $\text{Fe}^{2+}$ 、 $\text{H}_2\text{O}_2$  とメルカプトエタノールを加えると発光する。抽出液中に存在する発光物質を精製して純品を得たが、この物質はカタラーゼの一種であることが判明した。ただし、このカタラーゼがカタツムリ発光の発光物質であるという確証はまだ得られていない。

The snail *Quantula striata* is the only known example of luminous terrestrial gastropod (HANEDA, 1979). It was discovered in Singapore (HANEDA, 1946, 1955), and studies have been reported on the structure of light organ, the life history, and the *in vivo* luminescence (HANEDA, 1963, 1979, 1981; HANEDA and TSUJI, 1969).

Differing from various other bioluminescent organisms, the *in vivo* luminescence of *Q. striata* can not be elicited by means of artificial stimulation. In addition, there is no known way to recover the luminescence of this snail once the specimens have been frozen. Those properties are unfavorable to the study of the chemical nature of the luminescent system.

In the preliminary stage of the present study, we have tried to elicit luminescence from the extracts of frozen specimens that were prepared in various manners, singly or in combinations of the extracts, by adding various chemicals; the efforts were made mainly based on the presently available knowledge on the chemistry of bioluminescence systems. We have found that luminescence can be elicited from aqueous extracts only when the extracts were treated with  $\text{Fe}^{2+}$ ,  $\text{H}_2\text{O}_2$  and a thiol compound, a combination of reagents which is new in bioluminescence. The purification and characterization of the active substance involved in this light emission are described in the present report.

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### Materials and methods

*Materials* The specimens of *Quantula striata* were collected in Singapore. The specimens were frozed alive and kept at  $-70^{\circ}\text{C}$  until used. Phenyl-Sepharose CL-4B, bovine liver catalase, aldolase and apoferritin were obtained from Pharmacia Inc., and Fractogel TSK HW-55(F) was purchased from EM Science.

*Assay of luminescence activities in extracts* To 3 ml of 10 mM MOPS buffer, pH 6.75, containing 1 mM EDTA plus an amount of luminescent substance which has a light-emitting capacity of less than  $5 \times 10^9$  photons, 30  $\mu\text{l}$  of 0.1 M  $\text{FeSO}_4$ , 50  $\mu\text{l}$  of 10%  $\text{H}_2\text{O}_2$  and 30  $\mu\text{l}$  of 1 M 2-mercaptoethanol were successively added at  $25^{\circ}\text{C}$ . The addition of the last component, 2-mercaptoethanol, triggered the emission of light. When the luminescence dimmed at about two minutes of reaction time, an additional amount of 1 M 2-mercaptoethanol (30  $\mu\text{l}$ ) was added to accelerate the completion of light-emission. The amount of light emitted was measured with a photomultiplier apparatus that had been calibrated with *Cypridina* bioluminescence (SHIMOMURA and JOHNSON, 1970).

*Extraction and purification of the luminescent substance* Eight frozen

Table 1. Purification of the luminescent principle of *Quantula*.

Step	Procedure <sup>1)</sup>	Protein amount, <i>a</i> (mg) <sup>2)</sup>	Luminescence activity, <i>b</i> (units) <sup>3)</sup>	Specific activity <i>b/a</i>
	Initial extract, from 8 specimens.		1650	
1.	Adsorption on Phenyl-Sepharose CL-4B column in 1 M $(\text{NH}_4)_2\text{SO}_4$ , followed by stepwise elution with decreasing concentration of $(\text{NH}_4)_2\text{SO}_4$ .	23	1340	58
2.	Gel filtration on Fractogel TSK HW-55(F) column (1.6 $\times$ 67 cm).	12	1040	87
3.	Anion exchange chromatography on DEAE cellulose column (1 cm $\times$ 6.5 cm), by linear increase of NaCl concentration from 0 to 0.5 M.	4.5	740	164
4.	Chromatography on Phenyl-Sepharose CL-4B column (1 cm $\times$ 3 cm), by elution with a concave gradient of concentration from 1.2 M $(\text{NH}_4)_2\text{SO}_4$ to pure water.	2.1	600	286
5.	High-performance liquid chromatography on Du Pont GF-250 size exclusion column (9.4 mm $\times$ 25 cm).	0.52	410	788

1) 10 mM Sodium phosphate buffer, pH 6.5, containing 2 mM EDTA was used throughout as the basic buffer; in Step 2 and Step 5, 0.2 M NaCl and 0.2 M sodium acetate, respectively, were included in the buffer. All at  $12^{\circ}\text{C}$ .

2) Estimated assuming that  $A_{1\text{cm}, 280}^{0.1\%}$  equals 1.0.

3) Total amount of light elicitable. One unit corresponds to  $10^{10}$  photons, assuming that the emission spectrum of the luminescence is similar to that of *Cypridina* luminescence. Measured at  $25^{\circ}\text{C}$ .

specimens of *Q. striata* (total weight about 10 g) were ground with 40 ml of 10 mM sodium phosphate buffer, pH 6.5, containing 2 mM EDTA in a mortar, then the mixture was centrifuged. The cake of insoluble matter was further extracted two times, each time with 40 ml of the buffer. The supernatants were combined and the luminescent substance therein was purified by five steps of chromatography, as summarized in Table 1. After steps 1, 3 and 4, the solutions of luminescent substance obtained were concentrated by saturating the solutions with  $(\text{NH}_4)_2\text{SO}_4$  and then collecting the precipitate of the luminescent substance by centrifugation.

### Results and discussion

The extracts of frozen specimens of *Quantula striata* emitted light only upon the additions of three kinds of chemicals, i.e.  $\text{Fe}^{2+}$ ,  $\text{H}_2\text{O}_2$  and a thiol compound; all of the three chemicals were essential for the emission of light. As to the thiol compound, 2-mercaptoethanol was more effective than dithioerythritol or cysteine. The luminescent principle of the extract was purified as summarized in Table 1. The purified product was apparently a protein judged by its properties revealed in the process of purification. The protein was practically pure according to a test by high-performance liquid chromatography (Fig. 1). The absorption spectrum was similar to the spectra of catalases (Fig. 2).

When bovine liver catalase was tested by the additions of  $\text{Fe}^{2+}$ ,  $\text{H}_2\text{O}_2$  and 2-mercaptoethanol in 1 mM EDTA buffer, pH 6.75, light was emitted and, moreover, the amount of light emitted was almost the same as the amount emitted from the purified protein on the basis of sample weight. Other hemoproteins, such as horseradish peroxidase and cytochrome c, did not emit light under the

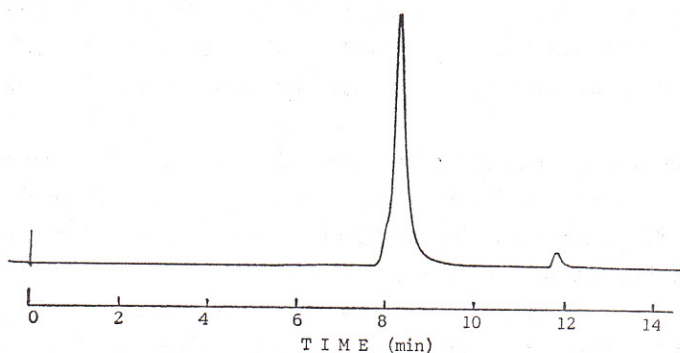


Fig. 1. Analytical high-performance liquid chromatography of the purified luminescent protein of *Quantula striata* on Du Pont GF-250 column (9.4 mm $\times$ 25 cm) with 10 mM sodium phosphate buffer, pH 6.5, containing 2 mM EDTA and 0.2 M sodium acetate at a flow rate of 1 ml/min., monitored by absorption at 280 nm, at 25°C. Samples were injected at 0 min. The small peak at 11.9 min was caused by salts.

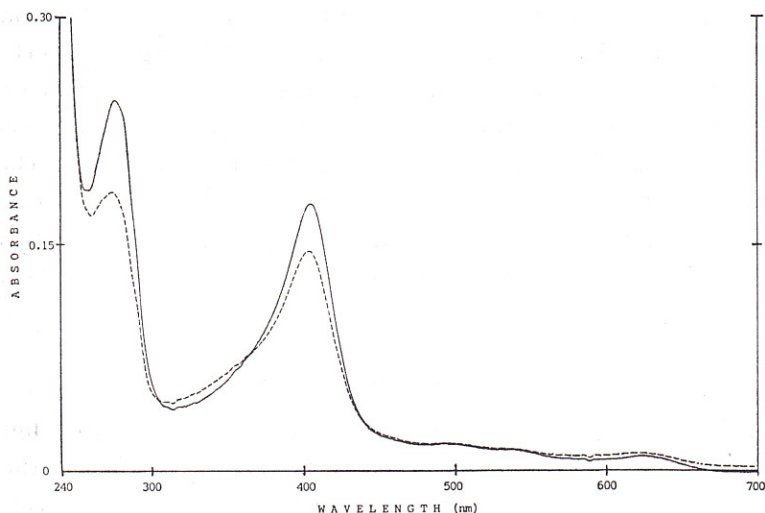


Fig. 2. Absorption spectra of the purified luminescent protein of *Quantula striata* (solid line) and bovine liver catalase (broken line) in the same buffer as used in the chromatography of Fig. 1.

same conditions. Activities of decomposing  $H_2O_2$  were not measured quantitatively. According to visual observation of evolved  $O_2$  gas, however, the purified protein and bovine liver catalase were nearly comparable in the catalase activity.

The evidences described above suggest that the purified luminescent protein is probably a catalase. The molecular size of the protein is slightly larger than that of bovin liver catalase on the basis of size-exclusion chromatography carried out under the same conditions as employed in the experiments of Fig. 1; the luminescent protein eluted at 8.41 minutes whereas bovine liver catalase eluted at 8.88 minutes. The calibration of the column with aldolase ( $M_r$  158000), bovine liver catalase ( $M_r$  232000) and apoferritin ( $M_r$  443000) indicated the molecular weight of the *Q. striata* luminescent protein to be approximately 260000.

Little is known at present on the mechanism of light emission that takes place when  $Fe^{2+}$ ,  $H_2O_2$  and a thiol compound are added to the purified protein or a catalase. The components involved in the reaction are highly antagonistic;  $H_2O_2$  quickly oxidizes thiol compounds in the presence of  $Fe^{2+}$  (JOCELYN, 1972), the purified protein and catalases catalyze the decomposition of  $H_2O_2$ , and the luminescence activity of the purified protein is diminished by thiol components in the presence of  $Fe^{2+}$ . Such antagonistic reactions would almost certainly decrease the quantum yield as well as the reaction rate of the luminescence reaction. According to the data given in Table 1, the quantum yield of the purified protein (Step 5) is estimated at approximately 0.34%. The quantum yield should be considerably greater than this value if the antagonistic reaction were

absent.

Despite the fact that the purified protein does luminesce, there is no direct proof showing that this luminescence reaction is actually the *in vivo* bioluminescence reaction of *Q. striata*; establishing it will require careful chemical studies on the live, luminous specimens, especially on their light organs. Designating the purified protein a photoprotein (SHIMOMURA and JOHNSON, 1966; SHIMOMURA, 1985) or a bioluminescent protein would not be justified until this point is established.

The present results show that catalases can cause luminescence. Considering that catalases are widely distributed in animals, plants and fungi, bioluminescence systems involving catalases might occur in some of the many bioluminescent organisms that have not been chemically examined, in addition to the snail *Q. striata*. An intriguing case was recently reported by ZINNER and NASSI (1985) on the bombardier beetle *Branchinus*, in which the *in vivo* defensive reaction involving a catalase is possibly bioluminescent.

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