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BIOSYNTHESIS OF CYPRIDINA LUCIFERIN IN *CYPRIDINA NOCTILUCA*

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Abstract – Luminescence in the marine ostracods *Cypridina noctiluca* and *Vargula hilgendorfii* is produced by Cypridina luciferin-luciferase reaction. In this study, a feeding experiment with stable isotope-labeled amino acids was performed in *C. noctiluca*. The results of LC/ESI-MS analyses indicated that *C. noctiluca* is able to synthesize Cypridina luciferin *de novo* from three amino acids (L-tryptophan, L-isoleucine, and L-arginine).

Luminescence in living animals is produced principally through luciferin-luciferase reaction.¹ In marine organisms, the chemical structures of several luciferins have been determined, including coelenterazine,² *Watasenia* luciferin,³ Cypridina luciferin,⁴ krill luciferin,⁵ and dinoflagellate luciferin.⁶ The well-characterized luciferins are imidazopyrazinone (3,7-dihydroimidazopyrazin-3-one) compounds such as coelenterazine and Cypridina luciferin, and they are distributed in various marine animals.^{1,7,8,9} Luminous ostracods have been found in the genera of *Vargula*, *Cypridina*, *Pyrocypris* and *Conchoecia*.^{1,10,11} *Vargula*, *Cypridina* and *Pyrocypris* are classified in the family Cypridinidae and the luciferin for luciferase reaction is Cypridina luciferin.¹⁰ *Conchoecia* is classified in the family Halocyprididae and the luciferin for luciferase reaction is coelenterazine.^{8,9,12} As previously proposed,^{4,13} imidazopyrazinone-type luciferins would be biosynthesized from three amino acids (i.e., arginine, isoleucine and tryptophan for Cypridina luciferin; phenylalanine and two tyrosines for coelenterazine). Recently, we found that Cypridina luciferin in *Vargula hilgendorfii* (formerly *Cypridina hilgendorfii*) was biosynthesized from three L-amino acids (L-arginine, L-isoleucine and L-tryptophan; Figure 1).¹⁴⁻¹⁶ However, the biosynthesis of Cypridina luciferin in other species such as *Cypridina* and *Pyrocypris* has

not been reported. In this study, we quantified Cypridina luciferin in *Cypridina noctiluca* and determined its biosynthetic units by the methods of LC/MS.

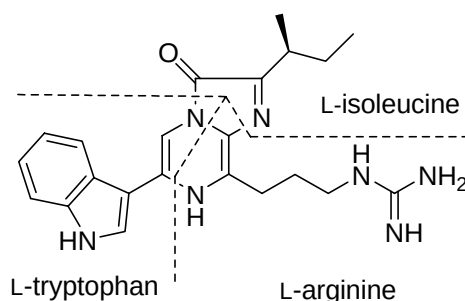


Figure 1. Chemical structure and biosynthetic units of Cypridina luciferin.

The specimens of *C. noctiluca* (ca. 0.7 mm of body length) were collected using a plankton-net (mesh size, 0.18~0.23 mm) at Shimoda, Shizuoka, Japan on 9 October 2005.

For quantification of Cypridina luciferin in *C. noctiluca*, three frozen specimens were extracted with 30 μ L of ethanol, and an aliquot (2 μ L) was analyzed by a reversed phase (RP)-HPLC connected to an electrospray-ionization (ESI) triple quadrupole tandem mass spectrometer (RP-HPLC/ESI-QqQ-MS/MS) on an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) equipped with API 2000 (Applied Biosystems, Foster City, CA). The RP-HPLC conditions were as follows: column, Cadenza CD-C18 (2 $\times$ 75 mm; Imtakt, Kyoto, Japan) connected to a Unison US-C18 pre-column (2 $\times$ 5 mm, Imtakt); mobile phase, methanol-water containing 0.1% formic acid, a linear gradient from 25% to 88% methanol for 7 min and then isocratic elution with 88% methanol for 3 min; flow rate, 0.2 mL/min; UV detection, 280 nm. The ESI-MS/MS mode was positive and the source temperature was 350°C. The authentic *dl*-Cypridina luciferin for the calibration was chemically synthesized,¹⁷ and was eluted at 8.3 min under the HPLC conditions described above. The amount of Cypridina luciferin was determined by monitoring the mass values of *m/z* 406 (parent ion) and 389 (fragment ion) using the multiple reaction monitoring (MRM) mode.¹² The results showed that the total amount of Cypridina luciferin in *C. noctiluca* was 17 to 46 pmol per specimen (n=3). This amount is comparable to the reported value for the congeneric species *Cypridina dentata* (13 to 28 pmol), which was estimated by luciferin-luciferase reaction.⁹ In *V. hilgendorffii*, the amount of Cypridina luciferin was estimated to be 75 pmol (ca. 1.0 mm of body length) to 7.8 nmol (ca. 3.0 mm) per specimen.

To demonstrate the incorporation of free amino acids into Cypridina luciferin in *C. noctiluca*, three stable isotope-labeled amino acids were used for the feeding experiments as follows: L-[indole-²H₅]tryptophan,¹⁴ L-[¹³C₆]isoleucine (Cambridge Isotope Laboratories, Inc., Andover, MA), and L-[guanidino-¹⁵N₂]arginine (Mass Trace, Inc., Woburn, MA). The composition of stable isotopes in the

labeled amino acids was determined by LC/ESI-ion trap (IT)-MS on an Agilent 1100 HPLC system equipped with an Esquire 3000 (ESI-IT-MS; Bruker Daltonics, Billerica, MA); mobile phase, 50% methanol-water containing 0.1% formic acid; flow rate, 0.2 mL/min (Table 1).

Table 1. Relative intensity of mass ion peaks (monovalent) in stable isotope-labeled amino acids

No. of stable isotope atom	Relative intensity (%)		
	L-[indole- ² H ₅]Trp	L-[¹³ C ₆]Ile	L-[guanidino- ¹⁵ N ₂]Arg
+0	1.07	1.19	1.47
+1	0.47	0.89	0.97
+2	0.85	0.26	100.00
+3	7.24	0.75	6.63
+4	38.53	1.91	0.53
+5	100.00	11.01	-
+6	11.19	100.00	-
+7	1.51	1.70	-

Feeding experiments were performed using a 24-well microplate. A stable isotope-labeled amino acid (25 mg) was dissolved in aqueous extracts of porcine liver (0.5 mL), and gelled with 3% agarose (Type VII, Sigma). The specimens (3~7 bodies) in a single-well fed with a piece of the gel for 10 days were frozen in liquid nitrogen, and then extracted with a 4-5 times weight volume of ethanol on dry ice. An aliquot (2 µL) was analyzed by LC/ESI-IT-MS under the conditions described above. The incorporation efficiencies of L-[indole-²H₅]tryptophan, L-[¹³C₆]isoleucine and L-[guanidino-¹⁵N₂]arginine into *Cypridina luciferin* were 15.4%, 2.8% and 2.4%, respectively (Table 2). These results suggested that *C. noctiluca* synthesizes *Cypridina luciferin* from these three amino acids.

Table 2. Incorporation of stable isotope-labeled amino acids into *Cypridina luciferin* (divalent ion) in *Cypridina noctiluca*

No. of stable isotope atom	Relative intensity (%)			
	Non-feeding	L-[indole- ² H ₅]Trp	L-[¹³ C ₆]Ile	L-[guanidino- ¹⁵ N ₂]Arg
+0	100.00	100.00	100.00	100.00
+1	25.29	29.30	26.00	24.69
+2	3.72	4.33	3.13	6.44
+3	0.68	2.31	0.64	0.86
+4	-	3.52	0.19	0.37
+5	-	11.55	-	-
+6	-	2.12	2.62	-
+7	0.29	0.58	1.18	0.77
Total	129.98	153.71	133.76	133.13
Incorporation*	-	15.44%	2.83%	2.37%

* Incorporation = (Total_{feeding} - Total_{non-feeding}) / Total_{feeding} × 100

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