Biosynthesis of luciferin in the sea firefly, Cypridina hilgendorfii: L-tryptophan is a component in Cypridina luciferin

ARTICLE *in* TETRAHEDRON LETTERS · MARCH 2002

Impact Factor: 2.38 · DOI: 10.1016/S0040-4039(02)00257-5

4 AUTHORS, INCLUDING:

Yuichi Oba
Nagoya University
79 PUBLICATIONS **1,691** CITATIONS

Pergamon

Tetrahedron Letters 43 (2002) 2389–2392

TETRAHEDRON LETTERS

Biosynthesis of luciferin in the sea firefly, *Cypridina hilgendorfii*: L-tryptophan is a component in Cypridina luciferin†

Yuichi Oba,[a,*] Shin-ichi Kato,[a] Makoto Ojika[a] and Satoshi Inouye[b]

[a]*Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa-ku, Nagoya 464-8601, Japan*
[b]*Yokohama Research Center, Chisso Co., 5-1 Okawa, Kanazawa-ku, Yokohama 236-8605, Japan*

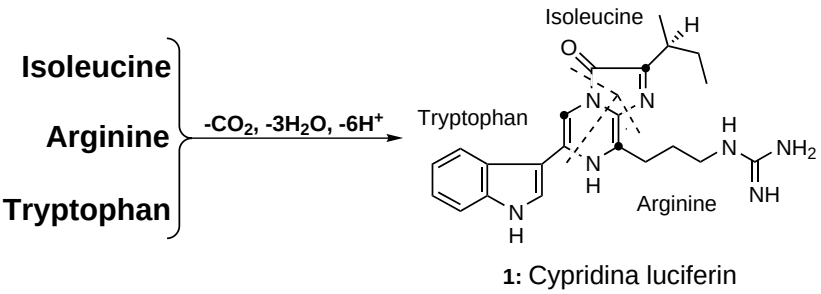
Received 7 January 2002; accepted 1 February 2002

Abstract—Feeding experiment to the marine ostracod crustacean, *Cypridina* (*Vargula*) *hilgendorfii*, using L-tryptophan labeled with deuterium at indole ring revealed that the labeled L-tryptophan was incorporated into Cypridina luciferin as a component and the animals were able to synthesize the luciferin in a de novo synthetic pathway. © 2002 Elsevier Science Ltd. All rights reserved.

The bioluminescence system in the marine ostracod crustacean, *Cypridina hilgendorfii* (presently *Vargula hilgendorfii*) living along Japanese coast, has been well investigated since Harvey reported the luciferin–luciferase reaction with the extracts of the animals in 1917.[1] The luciferin from *Cypridina hilgendorfii* (**1**: hereafter called Cypridina luciferin) was isolated[2] and the chemical structure was determined by studying the degradation products[3] and total synthesis[4] in 1966. On the other hand, *Cypridina* luciferase has been purified and its cDNA has been cloned[5] and used as a reporter protein in living cells.[6] On the basis of the chemical structure of Cypridina luciferin **1**,[3,4] the biosynthetic pathway of Cypridina luciferin has been proposed to be synthesized from three amino acids or their equivalents: arginine, isoleucine and tryptophan (or tryptamine) (Scheme 1).[3,4,7] However, no clear experimental evidence has been exhibited in living animals.

Here we reported the feeding study in vivo using the deuterium-labeled L-tryptophan **2** as an expected component of Cypridina luciferin and identified the incorporation of the deuterium atoms in L-tryptophan into Cypridina luciferin by LC/ESI-TOF MS analyses.

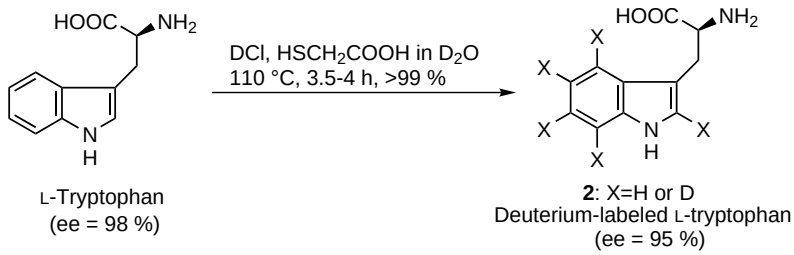
The synthesis of **2** was accomplished by the deuterium-exchange method with D_2O and DCl (Scheme 2). Briefly, L-tryptophan (300 mg, Wako Pure Chemical Industries, Ltd.) was dissolved in 2.0 ml of D_2O (99.9 atom% D, Cambridge Isotope Laboratories, Inc.) containing 0.4 ml of DCl (20 wt% solution, 99.5 atom% D, Aldrich) and 0.1 ml of mercaptoacetic acid (Aldrich).



Scheme 1. Structure of Cypridina luciferin **1** and its predicted biosynthesis. The closed circle represents the α-carbon of arginine, isoleucine and tryptophan.

Keywords: bioluminescence; biosynthesis; imidazopyrazinone; isotopic labeling; ostracod.
* Corresponding author. Tel./fax: +81-52-789-4284; e-mail: oba@agr.nagoya-u.ac.jp
† This paper is dedicated to the late Professor Hideshi Nakamura (deceased 9th November 2000).

PII: S0040-4039(02)00257-5



Scheme 2. Preparation of deuterium-labeled L-tryptophan **2**.

The mixture was degassed three times in a methanol-dry ice bath, and was heated in an oil bath at 110°C for 3.5–4 h. After removing mercaptoacetic acid by the extraction with ethyl acetate, the reaction mixture was evaporated and this labeling procedure was repeated. The resultant solution was dried up by evaporation, resolved in 2.0 ml of H_2O and dried up again. The white powder of **2** (360.1 mg) was obtained as a hydrochloride form and its yield was >99%, which was determined by using the data of the deuterium content of **2** from ESI-TOF-MS analysis as described below. The purity of **2** was determined by reversed phase-HPLC conducting on a Jasco 1500 system equipped with a Develosil ODS-SR-5 column (4.6×250 mm; Nomura Chemicals) and a multiwavelength detector (195–650 nm; MD-2010 plus, Jasco). Any products except **2** were not detected in this analysis (data not shown). The absolute configuration of tryptophan was determined by the method of Marfey.[8,9] The optical purity of L-tryptophan used for the labeling was 98% ee and the deuterium labeled **2** was 95% ee with the L-configuration. Further, to confirm the number of deuterium atoms in **2**, ESI-TOF-MS analysis was carried out with a Mariner Biospectrometry Workstation (Applied Biosystems). The results exhibited that $[D_4]$- and $[D_5]$-L-tryptophan (m/z 209 and 210 ($M+H^+$), respectively) were most abundant (Fig. 1) and the peak ratio of $[D_4]$- and $[D_5]$-L-tryptophan was 40:100.

Specimens of *C. hilgendorfii* were collected at Mukaishima, Hiroshima in Japan on 27 Sept. 2001, and were kept in an aquarium. For the feeding experiments, the deuterium-labeled **2** (50 mg) dissolved in a water-extract from the porcine liver was gelled in 3% agarose (Type VII: Sigma) as the food and was fed to the animals in a small partition of the aquarium at 22°C. After 6 days feeding, the animals were quickly frozen by liquid nitrogen. For extraction of Cypridina luciferin, six frozen animals (wet weight; 41.6 mg) were homogenized in 0.12 ml of ethanol by a micropestle on an ice bath and centrifuged at 12,000× g for 10 min. The resultant supernatant was filtrated using an Ultrafree-MC 0.45 μm filter (Millipore), and then was analyzed by LC/ESI-TOF-MS with an Agilent 1100 HPLC system (Hewlett–Packard) connected to a Mariner Biospectrometry Workstation.[10] The retention time of the extracted Cypridina luciferin on HPLC (asterisk in Fig. 2) was identical with that of the chemically synthesized *dl*-Cypridina luciferin as a standard.[10,11] Mass spectrum of the separated Cypridina luciferin showed the significant $[D_5]$-luciferin peak (m/z 410 (M^+), Fig. 3), compared to the control luciferin that was extracted from the animals without feeding. The peak ratio of $[D_4]$- and $[D_5]$-luciferin was 40:100 which was identical to that of the labeled $[D_4]$- and $[D_5]$-L-tryptophan as a feeding source. The labeling efficiency of **2** was 9.2% of the total luciferin in vivo. This evidence strongly indicated that L-tryptophan was one of the components in the Cypridina luciferin biosynthesis (Scheme 3). *C. hilgendorfii* is the first example that the animals can produce the imidazopyrazinone-type luciferin from free amino acid by a de novo biosynthetic pathway in marine organisms including jellyfishes[12], shrimps[13] and fishes.[14]

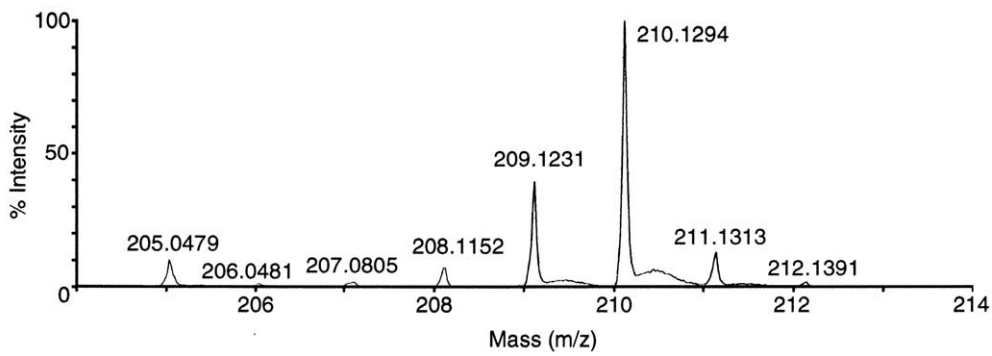


Figure 1. Mass spectrum of deuterium labeled L-tryptophan **2**.

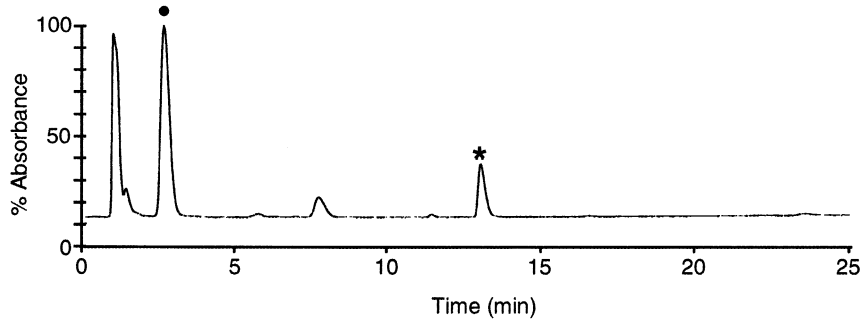


Figure 2. HPLC analysis of an ethanol-extracted fraction from *C. hilgendorfii* fed with **2**. The asterisk and the closed circle represent Cypridina luciferin and L-tryptophan, respectively.

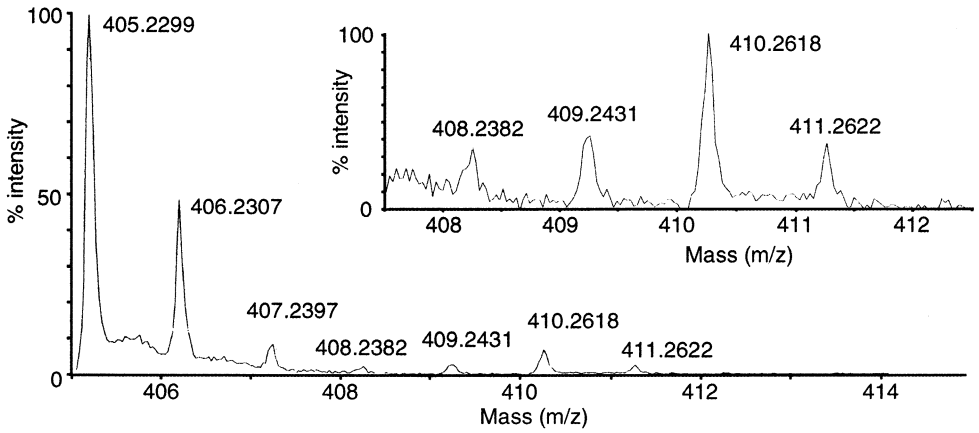
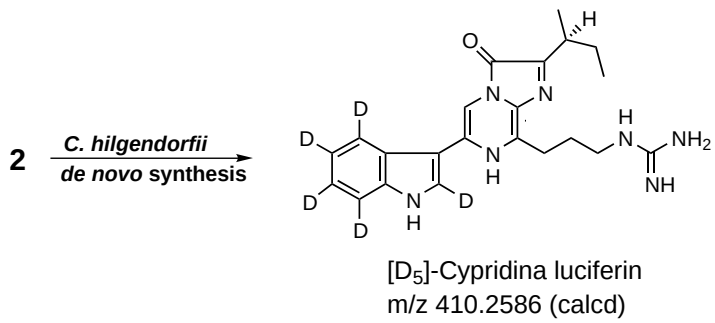


Figure 3. Mass spectrum of Cypridina luciferin isolated from *C. hilgendorfii* fed with **2**. Inset: magnification of the isotope peaks due to incorporation.



[D_5]-Cypridina luciferin
m/z 410.2586 (calcd)

Scheme 3. [D_5]-Cypridina luciferin synthesis from **2.**

Acknowledgements

We thank Dr. T. Uyama, Mukaishima Marine Biological Laboratory, Hiroshima University, Japan for collecting the animals. This work was supported in part by a Grant-in-Aid for Scientific Research on Priority Areas (A) from the Ministry of Education, Culture, Sports, Science and Technology.

References

1. Harvey, E. N. *Am. J. Physiol.* **1917**, *42*, 318–341.
2. Shimomura, O.; Goto, T.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1957**, *30*, 929–933.
3. Kishi, Y.; Goto, T.; Hirata, Y.; Shimomura, O.; Johnson, F. H. *Tetrahedron Lett.* **1966**, *7*, 3427–3436.
4. Kishi, Y.; Goto, T.; Inoue, S.; Sugiura, S.; Kishimoto, H. *Tetrahedron Lett.* **1966**, *7*, 3445–3450.
5. Thompson, E. M.; Nagata, S.; Tsuji, F. I. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 6567–6571.
6. Inouye, S.; Ohmiya, Y.; Toya, Y.; Tsuji, F. I. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9584–9587.
7. McCapra, F.; Roth, M. *J. Chem. Soc., Chem. Commun.* **1972**, 894–895.
8. Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.

9. Fujii, K.; Ikai, Y.; Oka, H.; Suzuki, M.; Harada, K. *Anal. Chem.* **1997,** 69, 3346–3352. After adjusting pH of a solution of tryptophan (2.5 μmol) to pH 9.0 with 1 M $NaHCO_3$, 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (3.6 μmol) was added and incubated at 37°C for 1 h, and then quenched with 1N HCl (20 μmol). Reversed phase HPLC conditions: column, Develosil ODS-SR-5 (4.6×250 mm); mobile phase, H_2O (0.1 M ammonium acetate, pH 3)-acetonitrile, 30–60% in 45 min; flow rate, 0.8 ml/min; monitor at 340 nm. Retention times of L- and D-derivatives were 25.8 and 28.3 min, respectively.
10. HPLC: column, a Cadenza CD-C18 (2.0×75 mm, Imtakt); mobile phase, H_2O (0.1% formic acid)–methanol, 25–75% in 25 min; flow rate, 0.2 ml/min; monitor at 280 nm. ESI-TOF-MS: positive mode, split ratio; 1:40 (5 μl/min).
11. Nakamura, H.; Aizawa, M.; Takeuchi, D.; Murai, A.; Shimomura, O. *Tetrahedron Lett.* **2000**, *41*, 2185–2188.
12. Haddock, S. H. D.; Rivers, T. J.; Robinson, B. H. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 11148–11151.
13. Thomson, C. M.; Herring, P. J.; Campbell, A. K. *J. Mar. Biol. Ass. UK* **1995**, *75*, 165–171.
14. Tsuji, F. I.; Barnes, A. T.; Case, J. F. *Nature* **1972**, *237*, 515–516.