Tetrahedron Letters No. 32, pp 2771 - 2774, 1974. Pergamon Press. Printed in Great Britain.

FIREFLY BIOLUMINESCENCE III.

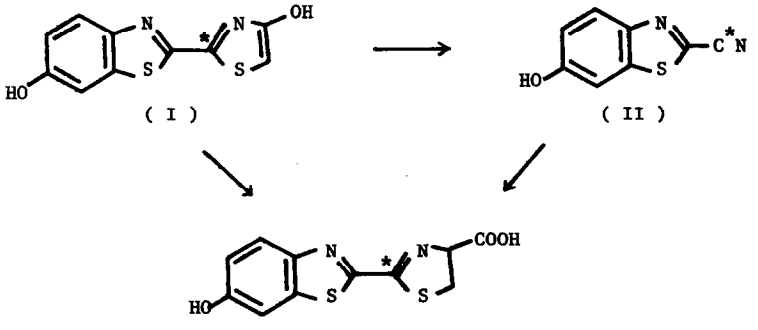
CONVERSION OF OXYLUCIFERIN TO LUCIFERIN IN FIREFLY

Kunisuke Okada*, Hideo Iio, Ichiro Kubota and Toshio Goto

Department of Agricultural Chemistry, Nagoya University, Chikusa, Nagoya 464 Japan

(Received in Japan 19 June 1974; received in UK for publication 25 June 1974)

After numerous efforts by a large number of investigators, it has become mostly possible to explain the phenomena of firefly bioluminescence concerning the detailed organic mechanism for overall reaction leading to light emission.[1] The extensive interest arising from the successful synthesis of firefly oxyluciferin (I)[2] led us to investigate further the chemical properties and biological roles of (I) in the firefly system. It was one of our recent results that oxyluciferin (I) has a strong inhibitory action on the firefly luciferase competitively to firefly luciferin (III).[3] In other words, the luminescence product, oxyluciferin, surpresses the turnover of firefly luciferase for the next light emission. Although many factors have to be considered to explain the flashing phenomena of firefly,[4] at least it must be operated in vivo that oxyluciferin is removed from and luciferin is supplied to the enzyme. In this point of view, we posturated the possibility of conversion of oxyluciferin to luciferin in vivo because of the most economical explanation for the above two terms. This communication shows the experimental support on this hypothesis.



First of all, C^{14}-oxyluciferin (I , * = C^{14}) was injected[5] to live fireflies, after several hours which were quickly frozen with dry ice and the crude luciferin was extracted by the usual procedure following dilution with 3 mg of cold luciferin. It was further purified as its diacetate derivative[6] to a constant radioactivity. In addition, we administrated C^{14}-2-cyano-6-hydroxybenzothiazole (II, * = C^{14}) to live fireflies as well. These data which indicate the in vivo formation of luciferin from oxyluciferin and even from the nitrile (II) were summarized in Table 1 (Experiments 1, 2 and 3).

Table 1. C^{14} Feeding Experiments using live and frozen Fireflies.

Expt.	Substrate	μg fed	Number of fireflies	Incubation[a] (hr)	Radiochemical yield[b] (%)
1	I	58.6[c]	16	1	0.160
2	I	58.6[c]	16	6	0.366
3	II	50.2[d]	15	3	1.630
4	I	50.0[e]	10	1	0.105
5	I	50.0[e]	10	3	0.143
6	I	50.0[e]	10	6	0.211
7	I[f]	50.0[e]	10	3	0.834
8	I	50.0[e]	10	3	0.168
9	I	50.0[e]	10	3	0.187
10	I	50.0[e]	10	3	0.164

a) The cell free extract used in Experiments 4 to 10 was prepared from frozen firefly lanterns by grinding with quartz powder in 5 ml of Tris buffer (pH 7.4, 0.01 M) followed by centrifugation at 1800 g. The supernatant mixed with C^{14}-oxyluciferin (50 μg in 100 μl of 0.125 N NaOH) and ATP (200 μg) was incubated at 29° after adjustment of the volume to 5 ml; b) Total radioactivity in isolated diacetate of luciferin divided by total radioactivity in the substrate multiplied by 100; c) 1.27×10^6 dpm/μmol; d) 1.33×10^6 dpm/μmol; e) 9.48×10^5 dpm/μmol; f) with 300 μg of cysteine.

On the other hand, oxyluciferin was also transformed into luciferin in similar ratios when it was incubated in a supernatant prepared from frozen firefly lanterns (Experiments 4, 5 and 6), in which cases luciferin thus formed was always accompanied by a small amount of II. But, the addition of cysteine into the incubation mixture extremely improved the radiochemical yield in luciferin (Experiment 7).

These findings coupled with the experiment 3 suggest the nitrile II could be an intermediate in the transformation of oxyluciferin into luciferin. This transformation, however, may not be catalyzed by enzyme, since the incorporation of I did not depend on the amount of the supernatant used (Experiments 8, 9 and 10). In fact, II could be isolated from the incubated solution of oxyluciferin itself even in a neutral buffer. Further experiments were done to find the best condition for non-enzymatic production of II from I . Figure 1 and 2 demonstrate the optimum pH and the final yields of II after prolonged incubation in degassed buffer, respectively.

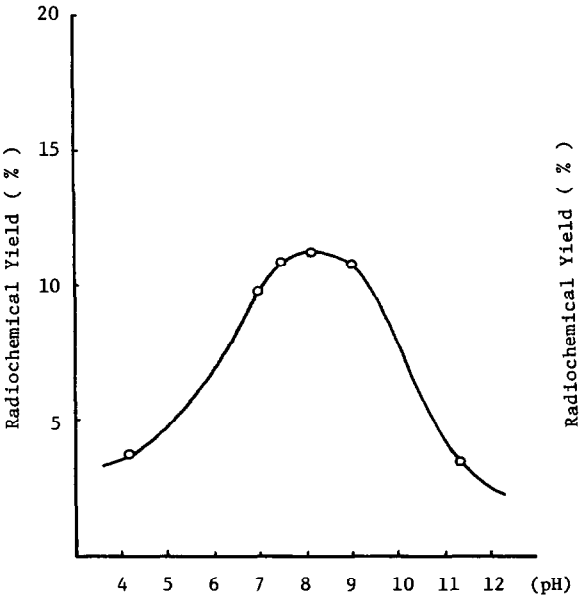


Fig. 1. Effect of the pH on the Radiochemical yield in 2-cyano-6-hydroxybenzothiazole from C^{14}-oxyluciferin (9.48×10^5 dpm/μmol) on incubation in 0.01 M buffer for 6 hr. at 29°.

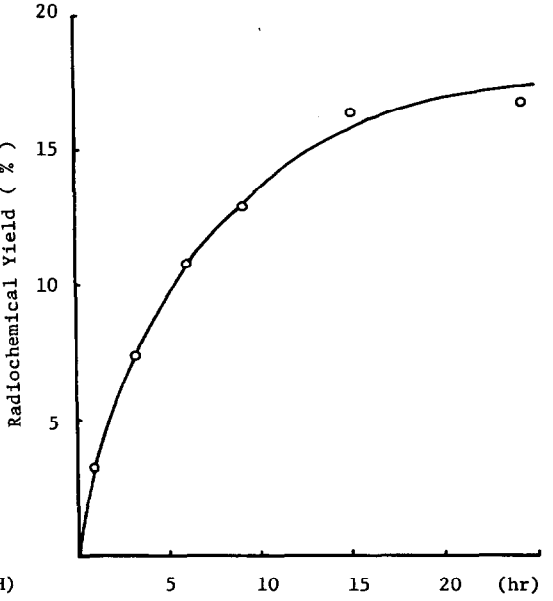


Fig. 2. The Radiochemical yield in 2-cyano-6-hydroxybenzothiazole from C^{14}-oxyluciferin (9.48×10^5 dpm/μmol) against incubation time at 29° in degassed buffer (pH 8.35, 0.01 M).

In summary, the incorporation of radioactivity of oxyluciferin (I) into luciferin (III) obtained from in vivo administration is possibly explained by the following two step reactions : 1) transformation of oxyluciferin to 2-cyano-6-hydroxybenzothiazole and 2) condensation of II with cysteine to yield luciferin.[7] These two reactions, which proceed in very mild conditions, could occur in firefly lanterns suggesting the possibility of conversion of oxyluciferin to luciferin in the organism.

REFERENCES

1) (a) T. A. Hopkins, H. H. Seliger, E. H. White and M. W Cass, J. Amer. Chem. Soc., 89, 7148 (1967); (b) F. McCapra, Y. C. Chang and V. P. Francois, Chem. Commun., 22 (1968); (c) E. H. White, E. Rapaport, T. A. Hopkins and H. H. Seliger, J. Amer. Chem. Soc., 91, 2178 (1969); (d) W. D. McElroy, H. H. Seliger and E. H. White, Photochem. Photobiol., 10, 153 (1969); (e) M. Deluca and M. E. Dempsey, Biochem. Biophys. Res. Commun., 40, 117 (1970); (f) E. H. White, E. Rapaport, H. H. Seliger and T. A. Hopkins, Bioorg. Chem., 1, 92 (1971); (g) W. D. McElroy and M. Deluca in Chemiluminescence and Bioluminescence, M. J. Cormier, D. M. Hercules and J. Lee, Ed., Plenum Pulb. Co., New York, N. Y., 1973, pp 285-311.

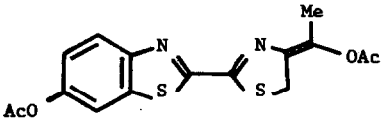
2) N. Suzuki, M. Sato, K. Okada and T. Goto, Tetrahedron, 28, 4065 (1972)

3) T. Goto, I. Kubota, N. Suzuki, Y. Kishi and S. Inoue in Chemiluminescence and Bioluminescence, M. J. Cormier, D. M. Hercules and J. Lee, Ed., Plenum Publ. Co., New York, N. Y., 1973 pp 325-355.

4) W. D. McElroy and H. H. Seliger in Bioluminescence in Progress, F. H. Johnson and Y. Haneda, Ed., Prinston Univ. Press, Prinston, N. J., 1966, pp 427-458.

5) The nitrile II was synthesized by displacement of 2-sulfonamido-6-methoxybenzothiazole with $KC^{14}N$ followed by demethylation by treatment with pyridine hydrochloride [E. H. White, F. McCapra and G. F. Field, J. Amer. Chem. Soc., 85, 337 (1963)]. The radioactive II thus obtained was condensed with ethyl thioglycolate to yield C^{14}-oxyluciferin, which was dissolved in 0.2 N NH_4OH just before use. The volume injected was 1 μl.

6) When treated with acetic anhydride and pyridine, luciferin was transformed to novel diacetate (i) in a good yield. Although luciferin is unstable towards light, the diacetate is reasonably stable and easily crystallized from methanol. The reaction may be similar to the Darkin-West reaction.



(i)

7) It was shown by E. H. White et al. that this reaction proceeds smoothly in a buffer of pH 8 at room temperature [E. H. White, F. McCapra and G. F. Field, J. Amer. Chem. Soc., 85, 337 (1963)].