EVIDENCE FOR THE IDENTITY OF THE LUMINESCENT SYSTEMS OF PORICHTHYS POROSISSIMUS (FISH) AND CYPRIDINA HILGENDORFII (CRUSTACEAN)

Milton J. Cormier, Jules M. Crane, Jr., and Yo Nakano
Department of Biochemistry
University of Georgia
Athens, Georgia 30601

Received November 8, 1967

One of the striking biological features of bioluminescence is its wide distribution throughout the phylogenetic spectrum of the animal kingdom. That is, the ratio of the number of luminous species to the total number of species is quite small, whereas the ratio of the number of phyla or classes containing luminous species to the total number of phyla or classes is quite large (Harvey, 1952).

During recent years the chemistry of bioluminescence has been examined in extracts of about 18 different species of bioluminescent forms (Cormier & Totter, 1964; Johnson & Haneda, 1966). The general observation has been that the luciferin and luciferase extracted from one organism differs in chemical structure from the luciferin and luciferase isolated from another species, especially if this species is not a member of the same family. That is, the luciferins and luciferases that originate from different species normally do not cross react to produce light. The only apparent exception to this that has been reported involves a cross reaction between a small crustacean, Cypridina, and two closely related species of shallow water marine fish, Apogon and Parapriacanthus (Johnson et al., 1960; Sie et al., 1961).

The difficulty in establishing the cross reaction between Cypridina and Apogon or Parapriacanthus as a true interphylum cross, is due to the fact that the luminous gland of these fish is connected via a duct to the gut. In addition, luminous Cypridina have been found in the gut of these animals (Johnson et al., 1961). Therefore, it is possible that the luciferin and luciferase

found in the luminous glands of these fish originate from ingested Cypridina.

However, we wish to report here that the luciferin and luciferase from a marine fish, Porichthys, obtained from the Gulf of Mexico, cross reacts with that of Cypridina. Unlike Apogon or Parapriacanthus, Porichthys contains hundreds of true luminescent photophores. These beautifully arranged organs are exclusively located within the dermis, are visible to the eye, and are complex structures consisting of photocyte cells, lens cells and a reflector layer (Harvey, 1952).

METHODS

Preparation of luciferin[1] - The photophores from 20-50 fish were excised from the animal, lyophilized, and extracted with 1000 ml of benzene for 24 hours under a purified hydrogen atmosphere using a soxhlet apparatus. The hydrogen was purified by passing over hot platinized asbestos. The extracted photophores were dried, in vacuo, overnight and re-extracted with 500 ml of methanol for 5 hours under a hydrogen atmosphere. The methanol solution, containing 50-75% of the extractable luciferin, was quickly evaporated to dryness in a Buchler flash evaporator. The residue was dissolved in about 2 ml of methanol and stored at -25°C. A precipitate formed that was removed by centrifugation at -20°C. This solution was further purified by thin layer chromatography under an argon atmosphere. The adsorbent was cellulose and the developing solvent was that of Tsuji (Tsuji, 1955). For comparative purposes in some experiments Cypridina luciferin was prepared the same way from dried Cypridina.[2]

Preparation of Porichthys Luciferase - Luciferase was extracted by grinding the freshly excised photophores, in the presence of distilled water or 0.05 sodium phosphate buffer, pH 6.9, with a Ten Broeck type tissue homogenizer. The debris was removed by centrifugation at 12,000 RPM for 10 minutes and the supernatant dialyzed vs 0.05M sodium phosphate buffer, pH 6.9 overnight. Some luci-

---

[1] Highly purified preparations of Cypridina luciferin and luciferase were generous gifts of Drs. F. H. Johnson and O. Shimomura, Princeton University.

[2] The authors wish to thank Dr. H. Seliger for a generous gift of dried Cypridina.

ferase activity was obtained by this procedure and was used in experiments described below. Numerous attempts to increase the luciferase yield were made but without success.

RESULTS AND DISCUSSION

Evidence for the identity of Porichthys and Cypridina luciferins - Chemical properties, such as auto-oxidizable characteristics, solubility, and Rf during chromatography, of both Porichthys and Cypridina luciferin are identical during purification. The Rf of each on thin layer chromatography, using cellulose as the adsorbent and Tsuji's solvent (Tsuji, 1955), is about 0.7.

Figure 1 shows that the kinetics of light production using either luciferin, is about the same when catalyzed by Cypridina luciferase. In this experiment the concentrations of both luciferins are adjusted to approximately the same level, as judged by total light measurements, and the kinetics of total light production then followed.

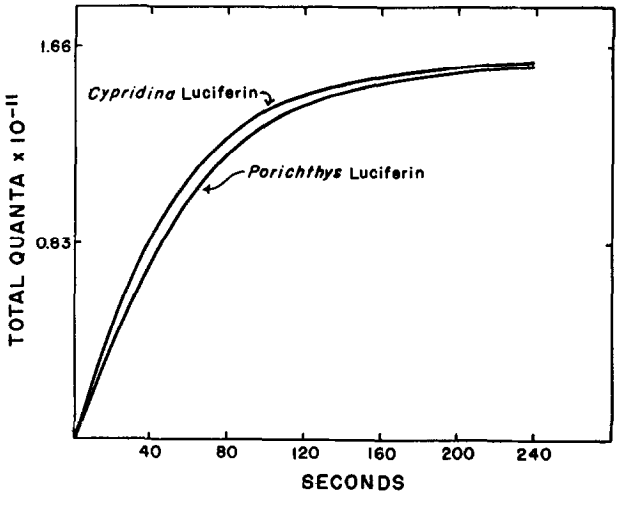

Figure 1. Kinetics of Cypridina luciferase-catalyzed light production using Cypridina vs Porichthys luciferin. 1 ml of 0.2 M sodium phosphate buffer, pH 7.0, which contained 0.03 ml of a dilute solution of Cypridina luciferase, was injected into an assay vial containing 0.01 ml of luciferin solution. An intergrating circuit was used for total light measurements.

When either Porichthys or Cypridina luciferin is mixed with dilute Cypridina luciferase solutions (not more than 10^{-3} mg/ml), a brilliant bluish luminescent flash is observed that requires dissolved O_2 and is easily visible to the non dark-adapted eye. At sufficiently low enzyme and high luciferin levels it is possible to obtain a steady state of sufficient duration to allow one to measure the spectral energy distribution of the light produced. Figure 2 compares the bioluminescent spectral energy distribution for the reaction catalyzed by Cypridina luciferase in the presence of O_2, and either Porichthys or Cypridina luciferin. As illustrated in Figure 2 the emission peak in either case is located at about 460 mμ and, furthermore, the half-bandwidths are also the same.

Porichthys luciferase will also react with Cypridina luciferin to produce light. However, the experiments indicate that the level of luciferase in Porichthys is extremely low. Thus it was impossible to do quantitative cross re-

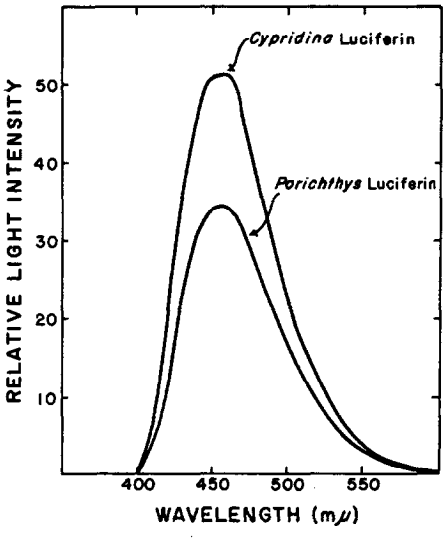

Figure 2. Spectral energy distribution of the Cypridina luciferase-catalyzed light reaction using Cypridina vs Porichthys luciferin. 0.1 ml of a methanolic solution of either luciferin was added to 1 ml of 0.2 M sodium phosphate buffer, pH 7.0, which contained 0.03 ml of a dilute solution of Cypridina luciferase. Emission as a function of wavelength was recorded with the use of an Aminco-Bowman Spectrophotofluorimeter (uncorrected for photomultiplier sensitivity).

actions with this enzyme. This low level of luciferase is consistent with visual observations on the bioluminescence of Porichthys. This animal produces a low level luminescence that persist for hours as contrasted to Cypridina which produces a brilliant and short-lived flash (Harvey, 1952).

The experiments described above suggest that Porichthys and Cypridina luciferins are either identical compounds or structurally very similar.

TABLE I
Relative luciferin content of the tissues and body fluids of Porichthys porosissimus

Tissue or Fluid	Percent of Photophore Emission		Order of Decreasing Luciferin Content	
	I	II	I	II
Photophores	100	100	1	1
Head "gels"	37	8.1	2	2
Subocular gel	18	6.2	4	3
Upper opercular gel	31	6.0	3	4
Hindgut contents	14	4.5	5	5
Skin	12	3.0	6	6
Liver	4.0	2.5	7	7
Blood	4.0	1.7	7	8
Spleen	2.9	1.4	9	9
Peritoneal fluid	---	0.88	-	10
Testis	3.3	0.82	8	11
Muscle	2.6	0.75	10	12
Urine	---	0.55	-	13
Eye	1.2	0.36	11	14
Bile	---	0.35	-	15
Endolymph	---	0.22	-	16

Tissue and fluid samples were removed from two specimens of Porichthys (I and II), weighed, and placed in 10 ml of cold methanol which was made 0.1N with HCl. The suspension was homogenized 3 minutes at 0°C, under a argon atmosphere, with a Servall Omni-Mixer and centrifuged at 0°C to remove suspended materials. 0.1 ml of the supernatant was assayed for activity as outlined in Figure 1 except that the height of the flash peak was used as a measure of relative luciferin concentration. At these light intensities the flash peak height is a linear function of the luciferin concentration. Light intensities obtained were converted to quanta sec^{-1} $gram^{-1}$ of tissue or fluid for comparative purposes.

Distribution of Porichthys luciferin within its tissues and body fluids - Table I shows that, although the largest concentration of luciferin occurs within the photophores of this animal, luciferin is widely distributed throughout its body. The "gels" around various parts of the head also contain a reasonable amount of luciferin. These are gelatinous tissues covered by a thin layer of connective

tissue and skin. In contrast, luciferin was not found in the tissues of Opsanus tau, a very close non-luminous relative of Porichthys.

The fact that reasonable amounts of luciferin are found in various tissues of Porichthys probably means that luciferin is either synthesized in the photophores and is leached out continuously via the circulatory system or that luciferin is synthesized in one or more tissues of the body, carried by the circulatory system, and stored in the photophores. Luciferase has been detected only in the photophores.

Concluding remarks - The bioluminescent cross reaction reported here between a crustacean and a fish would appear to have broad evolutionary implications for bioluminescence. Apparently, certain types of bioluminescent systems that occurred early in evolution have been maintained among fish, the most advanced evolutionary examples of bioluminescence.

Cormier and Totter (1964) found that certain patterns could be recognized among the known bioluminescent forms. For example, it was possible to group various bioluminescent systems into several major groups according to the type of biochemical reaction involved. It may be that only a relatively small number of biochemical types exist and that numerous cross reactions will be uncovered in the future. Thus Hori and Cormier (unpublished) have recently shown that the bioluminescent systems of Cavernularia and Renilla will also cross react.

ACKNOWLEDGEMENTS This work was supported in part by the National Science Foundation and the U. S. Atomic Energy Commission. One of us (M. J. Cormier) is a Career Development Awardee No. 1-K_3-GM-3331-01 of the U. S. Public Health Service.

References

Cormier, M. J. and Totter, J. R. Ann. Rev. Biochem., 33, 431 (1964).
Harvey, E. N., in "Bioluminescence", Academic Press, New York (1952).
Johnson, F. H. and Haneda, Y. (eds.). "Bioluminescence in Progress", Princeton University Press, Princeton, New Jersey (1966).
Johnson, F. H., Haneda, Y., and Sie, E. H-C. Science, 132, 422 (1960).
Johnson, F. H., Sugiyama, N., Shimomura, O., Saiga, Y., and Haneda, Y. Proc. Nat'l Acad. Sci., 47, 486 (1961).
Sie, E. H-C., McElroy, W. D., Johnson, E. H., and Haneda, Y. Arch. Biochem. & Biophys., 93, 286 (1961).
Tsuji, F. I., Chase, A. M., and Harvey, E. N., in The Luminescence of Biological Systems. F. H. Johnson (Ed.). AAAS, Washington, D. C., 1955.