CRYSTALLINE LUCIFERIN FROM A LUMINESCENT FISH, PARAPRIACANTHUS BERYCIFORMES*

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Biochemical research on luminescent enzyme-substrate ("luciferin-luciferase") systems has resulted thus far in the isolation and crystallization of the luciferin from only two types of luminescent organisms, namely, the firefly1 and the small ostracod crustacean, Cypridina.2 Synthesis of the former luciferin has very recently been achieved,3 and a tentative structure proposed for the latter luciferin,4 which is a considerably more complicated molecule. Both differ chemically from the diffusible factors involved in bacterial luminescence.5

The present study has resulted in crystallization of luciferin from one of the two examples of luminescent fishes; namely, Parapriacanthus beryciformes6 and Apogon (marginatus) elliotti,7 which have yielded crude aqueous extracts of luciferin and luciferase that emit light on mixing at room temperature. The essential components of the two systems react interchangeably, and those of Apogon have been shown to cross-react with the luciferin and luciferase of Cypridina.7 Present experiments have revealed that luminescent cross-reactions occur among all three of these systems.

A total of over 4,000 specimens of Parapriacanthus was collected at Tateyama,8 Japan, and brought, packed in dry ice, to the Tokyo Kyoiku University, where the small, superficially embedded, ventral light organs were grossly removed and ground in a mortar chilled with dry ice. A very small amount of crystalline luciferin was obtained from such brei by the procedure of Shimomura et al.2 as recently modified by Haneda et al. for crystallizing Cypridina luciferin,9 involving initial extraction with methanol at low temperatures (circa −20°C) and chromatographing a butanol solution of the de-fatted residue on an alumina column. A much larger quantity, amounting to about 20 mg of pure crystalline luciferin, was obtained by the same procedure from the pyloric caeca dissected from some 2,300 specimens (Fig. 1).

The anatomical and histological details of the caeca remain to be worked out, but they appear to be connected by a minute duct to the light organs, and they evidently store luciferin. The intact caeca are nonluminous, and likewise the brei resulting from grinding with water, but a brilliant luminescence results on addition of Cypridina luciferase to the brei. In the stomachs of about a dozen of the 2,300 specimens, a few individual Cypridina, dead but still luminous, were discovered. This fact, plus observation of a luminescent reaction of crystallized luciferin from the fish with purified luciferase of Cypridina, and chemical similarities found between the luciferins from the two different sources (see below) raises the question as to whether Parapriacanthus depends on ingested Cypridina as the chief or sole source of its luciferin. While this possibility cannot be rigidly excluded on the basis of present evidence, it would present a fantastic situation
Fig. 1.—Luciferin isolated from the pyloric caeca of *Parapriacanthus*, recrystallized one time and photographed in immersion oil by means of a 50 X, Wild verticolor phase lens. Magnification: X 1,200. Photomicrograph by L. I. Rebhan.

Fig. 2.—Absorption spectra of methanolic solutions of luciferin from *Cypridina* (hollow circles) and *Parapriacanthus* (solid circles) at 25°C.
from a biochemical and evolutionary point of view. Moreover, luminescent specimens of *Apogon*, whose light-emitting system anatomically does not differ greatly from that of *Parapriacanthus*, have been recently collected (by Y. H.) in areas of the Indian ocean where no specimens of *Cypridina* could be found.

The chemical properties of crystalline luciferin from *Parapriacanthus* and *Cypridina*, respectively, were found to be essentially identical in the following respects: (1) melting point, 185–195°C, not sharp, with evidence of softening and reddening at 170°C; (2) absorption spectra (Fig. 2), with E\textsubscript{1\textsuperscript{cm}} (25°C, 435 \textmu m) measured as 187.0 and 187.5 for *Cypridina* and *Parapriacanthus*, respectively, suggesting fundamentally the same structure of the chromophore, as well as molar equivalence, probably also molecular weight; (3) specific luminescence potency in the reaction with *Cypridina* luciferase, amounting to 4,640,000 L.U. (light units, on an arbitrary scale of integrated total light) per mg for *Cypridina* luciferin, and 4,810,000 L.U. for *Parapriacanthus* luciferin; (4) a positive Sakaguchi reaction, indicating the same structure at a mono-substituted guanidine in the two luciferins; (5) identical paper chromatograms of the two luciferins, using Tsuji’s solvent,\textsuperscript{10} modified to consist of ethyl acetate, butanol, ethanol, and water in a ratio of 3:2:2:3; and (6) identical paper chromatograms of the products of acid hydrolysis (4 N. HCl, 15 hours at 120°C in air) and alkaline hydrolysis (10% barium hydroxide, 15 hours at 120°C in air), the former revealing the presence of isoleucine and 4-guanidino butyric acid, and the latter, the presence of isoleucine and 4-aminobutyric acid, using in each case a developing solvent consisting of butanol, acetic acid, and water in the proportion 4:1:1.

Evidence for chemical identity or near-identity of these luciferins is not surprising in view of their cross-reactions with the opposite luciferases, though this phenomenon is virtually unique among the luciferin-luciferase systems which have been separated from about a dozen different types among the numerous luminescent organisms known.\textsuperscript{11} While the significance from the viewpoints of comparative biochemistry and evolution remains to be elucidated, the foregoing results are interesting in constituting the third example of crystalline luciferin that had been isolated from a luminescent organism.

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3 W. D. McElroy, personal communication.


THE IDENTIFICATION OF A RIBOSOMAL-BOUND β-GLUCOSIDASE*

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Considerable evidence has accumulated in recent years to support the concept that ribosomes are the primary sites of protein synthesis. This view was originally supported by experiments in vivo and in vitro with C\(^{14}\) amino acids demonstrating that proteins pass through ribosomes during the course of their synthesis (see review by Novelli\(^1\)). More recently, not only has a nascent precursor of soluble proteins been recognized on ribosomes,\(^2\)\(^4\) but also specific protein\(^5\)\(^7\) synthesis has been obtained with in vitro experiments in which the specificity for protein synthesis resides in the ribosomes.

Similar evidence has recently been reported for yeast. Protein synthesis is associated with particulate RNA.\(^8\) Analysis of the ribosome fraction from exponentially growing yeast has revealed one major component having a sedimentation coefficient of 80S.\(^9\)\(^10\) In experiments with intact cells, C\(^{14}\) amino acids and S\(^{35}\) are incorporated first into ribosomal protein\(^11\)\(^12\) and can be chased from these to the soluble proteins.\(^12\) Webster\(^13\) has demonstrated in vitro that amino acids are incorporated into ribosomes in a manner analogous to that of mammalian and plant systems. Finally, the classes of S\(^{35}\)-labeled proteins released from ribosomes are identical to the classes of soluble proteins present in the cytoplasm.\(^14\)

The essential feature to emerge from the above findings is that ribosomes are involved in determining the amino acid sequence for each particular enzyme. One would therefore expect that at any given moment a fraction of these particles would still be associated with newly synthesized enzyme. A measure of the number of these ribosomal-bound enzymes per cell might therefore provide a minimal estimate of the number of specific enzyme-forming sites (ribosomes) for a given enzyme. In the present communication, we have undertaken an examination of ribosomal particles from a yeast constitutive for β-glucosidase. The preparation of ribosomal-bound enzyme, a description of its properties, and an estimate of its cellular distribution are given.

Materials and Methods.—A diploid yeast (Saccharomyces dohshanskii 1974 × Saccharomyces fragilis 110), constitutive for β-glucosidase synthesis, was grown aerobically at 30°C in synthetic