Inhibition of Photosynthesis in Some Algae by Extreme-Red Light

Abstract. Photosynthesis produced by far-red light (about 700 mμ) is reversibly inhibited in some algae by extreme-red light (~ 750 mμ).

In earlier communications from this laboratory (*1*) it was reported that illumination of a suspension of the red alga *Porphyridium cruentum* with far-red light, obtained by filtering light from an incandescent lamp through a very dense aqueous solution of the phycobilins, produces little or no photosynthesis. Yet this light contains wavelengths above 660 mμ, a marked fraction of which are absorbed by chlorophyll *a*. (The absorption band of this pigment extends in vivo to about 720 mμ.) If bands 10 mμ wide obtained with a monochromator are used for illumination, measurable rates of photosynthesis are obtained at all wavelengths up to 700 mμ. Furthermore, an "Emerson effect" can be observed in phycobilin-filtered light: if orange light is added to it, the resulting rate of photosynthesis is higher than in the orange light alone. This confirms the observation that chlorophyll *a* is markedly excited by the phycobilin-filtered light.

These seemingly conflicting results can be explained by the assumption that the band obtained by the use of the phycobilin filter contains extreme-red (or near-infrared) light (> 700 mμ), which inhibits photosynthesis caused by far-red light.

In order to check this hypothesis, algal suspensions were illuminated with a far-red band isolated from the light of an incandescent lamp by a Farrand interference filter No. 1322, which has a transmission peak at 700 mμ but transmits some light down to 680 mμ. We made sure that the intensity of this light was far below that which causes saturation of photosynthesis. After photosynthesis in this far-red light had been measured, extreme-red light, with a band width of about 10 mμ centering at 750 mμ, was added and photosynthesis was measured again. The results of these experiments are shown in Table 1.

Table 1. Inhibitory effect of extreme-red light (750 ± 10 mμ) on photosynthesis of *Porphyridium cruentum* in far-red light (~700 mμ).

Experiment No.	Rate of photosynthesis (μl of O_2 per hour)		Rate in combined light (in % of rate in far-red light alone)
	In far-red light	In far-red light plus extreme-red band	
1	4.01	2.23	56
2	1.87	0.29	15
3	1.83	0.29	16
4	3.30	2.04	62
5	1.08	0.84	78

The scattering of the results in column 4 of Table 1 may be due to small unintentional variations in the culturing of the cells. Despite this scattering, it is evident that the rate of photosynthesis in far-red light is consistently and significantly reduced by the addition of light of 750 mμ wavelength. This effect is completely reversible.

Porphyridium cruentum must thus contain a pigment which absorbs in the extreme-red at about 750 mμ and which, when excited, inhibits the photosynthesis caused by far-red light. This pigment must be present in small amounts, since no band is recognizable in the absorption spectrum of *Porphyridium* in the region of 750 mμ.

Preliminary results indicate that a similar effect occurs in the green alga *Chlorella* but apparently not in the blue-green alga *Anacystis*. It remains to be seen whether it occurs also in higher plants (*2*).

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2. This work was carried out under a grant (G4969) from the National Science Foundation.

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An Interphylum Luciferin-Luciferase Reaction

Abstract. The light-emitting enzyme-substance systems, heretofore separated from different types of luminescent organisms, exhibit a marked biological specificity and comprise chemically different components. Extracts from a luminescent fish are now found to cross react with a crustacean system; some properties of the new system and implications of the phenomenon are discussed.

Separate extracts, containing a relatively heat-stable (luciferin) and a heat-labile (luciferase) component, which react with the emission of visible light in vitro (the luciferin-luciferase reaction), have been obtained from not quite a dozen types of the multitude of luminescent organisms known. They have been obtained thus far from certain bivalves [*Pholas* (*1*), *Rocellaria* (*2*)], elaterid [*Pyrophorus* (*3*)] and lampyrid [*Photinus, Photuris, Luciola* (*4, 5*)] fireflies, ostracod crustacea [*Cypridina* (*6*), *Pyrocypris* (*7*)], a marine polychaete worm [*Odontosyllis* (*7*)], decapod shrimps [*Systallaspis* (*8*) *Heterocarpus* (*9*)], a fresh water limpet [*Latia* (*10*)], luminous bacteria [*Achromobacter, Photobacterium* (*11*)], a protozoan [*Gonyaulax* (*12*)], fish [*Parapriacanthus* (*13*), *Apogon* (*14*)], luminous fungi [*Collybia, Armillaria* (*15*)], and a pennatulid [*Renilla* (*16*)]. Numerous tests, over the past several decades, for light emission in cross reactions between components of different luminous organisms have indicated that the luciferins and luciferases are specific for a given type, definite cross reactions being rarely found (*17*), and then only with extracts of organisms rather closely related, such as two genera of ostracods (*7*) or two families of fireflies (*4, 18*). Moreover, among the three biochemically best known systems, namely, those of *Cypridina*, the firefly, and luminous bacteria, the diffusible factors are all chemically different and noninterchangeable, except for oxygen, which is required by each (*17*).

These facts have led to the view that, in general, the luciferin and luciferase of one type of luminescent organism are quite different from those of another type (*17, 19*), a view that accords not only with profound differences in emission spectra (*17, 20*) but also with the utterly random occurrence of bioluminescence on the phylogenetic scale of animals from protozoa to fishes as well as among bacteria and higher fungi. Experiments reported in this paper (*21*), however, favor a modification of this view, according to the following evidence.

Qualitative observations in regard to the recently discovered example of a luciferin-luciferase reaction in crude extracts of the photogenic organs of a fish, *Parapriacanthus* (*13*), indicated a reciprocal cross reaction with the luciferin and luciferase in crude extracts from a second luminescent fish, *Apogon* (*14*), representing a different family. The *Apogon* system was then found to exhibit an astonishing cross reaction with that of the crustacean, *Cypridina*. No visible evidence, however, was found of a cross reaction between the *Parapriacanthus* and *Cypridina* systems, nor between *Apogon* and the Japanese firefly *Luciola*, or the clam *Pholas*. Since purely visual observations with crude extracts have occasionally proved misleading, quantitative studies and experiments with some highly purified extracts of *Apogon* and *Cypridina* have been carried out, with convincing results.

Quantitative data of cross reactions in the *Apogon* and *Cypridina* systems are illustrated in Fig. 1. *Apogon* has one anterior and two posterior photogenic organs whose luciferin and lucif-

erase react with each other as well as with those of *Cypridina*, though not with components extracted from non-photogenic tissues of the fish. With a given enzyme concentration, the total light emitted is proportional to the amount of luciferin added. With a given concentration of luciferin, the rate of decay of luminescence is proportional to the enzyme concentration, except when the luciferin is in great excess, as it appears to be in the *Apogon* organ. Thus, when either a freshly excised or desiccated organ (the latter weighing about 1 mg) is ground and suspended in 1 or 2 ml of water, luminescence may persist for hours. If greatly diluted *Cypridina* luciferase is added early to this reaction mixture, a bright luminescence of short duration occurs. Evidently, *Apogon* luciferase is either less active than that of *Cypridina* toward

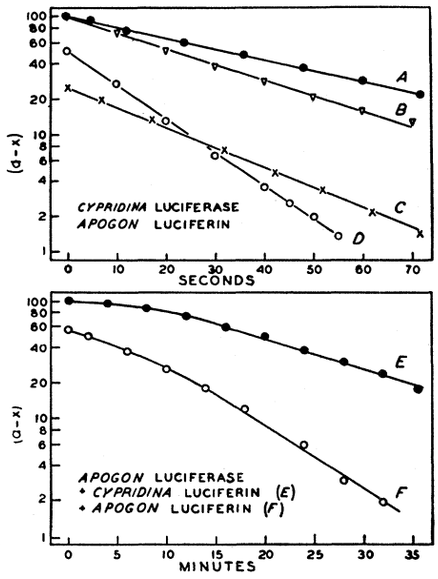

Fig. 1 Luminescence in cross reactions between the luciferin and luciferase of *Apogon* and *Cypridina* in phosphate-buffered solutions at neutral *p*H and room temperature. The data are plotted in the manner of a reaction first order with respect to the amount of luciferin, which is taken proportional to the total light, *a*, measured in arbitrary units by a photomultiplier integrating device, *x*, representing the fraction of the total light emitted up to a given time as indicated on the abscissa. Reaction mixtures were as follows: (*A*), *Cypridina* luciferase + anterior *Apogon* luciferin; (*B*), *Cypridina* luciferase, concentration 1, + posterior *Apogon* luciferin, concentration 1; (*C*), *Cypridina* luciferase, concentration 1, + posterior *Apogon* luciferin, concentration ¼; (*D*), *Cypridina* luciferase, concentration 2, *Apogon* luciferin, concentration ½; (*E*), anterior *Apogon* luciferase + *Cypridina* luciferin; (*F*), anterior *Apogon* luciferase + anterior *Apogon* luciferin. Differences in total light and slopes of only curves (*B*), (*C*), and (*D*) are significant.

the luciferin of either organism, or else it is present in only minute amounts. Efforts to concentrate it have been only partially successful, because of the presence of inhibitors which become simultaneously more concentrated. A relatively low activity of luciferase along with an excess of luciferin, resulting in a long-lasting, relatively dim luminescence, is possibly of survival value to the fish.

The photogenic organs of *Apogon* communicate with the gut, but are histologically and morphologically distinct (*22*). Careful examination of the stomach and intestinal contents of over 40 specimens have revealed no remains of small crustacea, such as *Cypridina* or related organisms. (These would not be expected from the habitat of the fish at a depth of 40 fathoms.) Thus the cross reactions cannot reasonably be attributed to the presence of extraneous, crustacean material such as was once responsible for the seeming luminescence of a flying fish (*23*).

The purification and properties of the *Apogon* system have been investigated and will be reported in detail elsewhere. It is worth noting here, however, that none of the diffusible factors known to function in other systems (*17*) have been found to increase the light of the *Apogon* system (*24*). The absorption spectra of chromatographed luciferins of *Apogon* and *Cypridina* have points in common, but are not identical, and the changes on oxidation are not the same. Both luciferins are associated with a yellow color by ordinary light and yellow fluorescence by ultraviolet light. The two luciferins are qualitatively alike in behavior during Anderson's (*25*) purification procedure, in undergoing an initially reversible, dark oxidation by oxygen or ferricyanide (*26*), in varying sensitivity to cyanide (*27*) at corresponding stages of purification, and in having nearly the same Rf on paper with Tsuji's (*28*) solvent. Under the conditions employed, the optimum *p*H for total light is associated with the substrate, being close to 7.0 when either enzyme is used with *Cypridina* luciferin, and between 6.3 and 6.5 with *Apogon* luciferin, while the optimum *p*H for rate of light emission is correlated with the specific enzyme, being from 7.6 to 7.8 with *Cypridina* luciferase, and about 7.2 with *Apogon* luciferase, using either of the two luciferins.

The enzymes differ in that *Cypridina* luciferase is sensitive to $4 \times 10^{-4}M$ versene, whereas *Apogon* luciferase is not, and in that *Cypridina* luciferase withstands exhaustive dialysis, whereas *Apogon* luciferase progressively loses activity in a manner that can be initially restored by the addition of dialyzate.

Although the similarities in the luminescent systems of a fish and crustacean could represent a rare, evolutionary coincidence, they as likely indicate that more of a thread of unity exists in the comparative biochemistry of luminescence among diverse types of organisms than has been hitherto supposed.

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