# Particulate Bioluminescence in Dinoflagellates: Dissociation and Partial Reconstitution

Abstract. *With the same extraction conditions used for* Gonyaulax polyedra, *soluble and particulate bioluminescence can be isolated from two additional species,* Pyrodinium bahamense *and* Pyrocystis lunula. *We have been able, for all three species, to dissociate soluble luciferin and luciferase from the particulate system. Luciferin can be incorporated into both reacted and unreacted particulate systems.*

Two distinct in vitro bioluminescent systems have been reported for the marine dinoflagellate *Gonyaulax polyedra.* A soluble system consisting of luciferase (enzyme), luciferin (substrate), and oxygen requires high salt concentrations and exhibits slow kinetics of light emission (*1*). A particulate system, named scintillons by DeSa *et al.* (*2*), emits a single flash as the result of an increase in hydrogen ion concentration (optimum *p*H 5.7); the rate of light emission approaches the fast kinetics of the in vivo flash observed when mechanical stimulation is given.

Under the same extraction conditions used for *G. polyedra*, soluble and particulate systems can be isolated from *Pyrodinium bahamense* and *Pyrocystis lunula.* Soluble luciferin and luciferase can be dissociated from the particulate systems of all three species. Luciferin can be incorporated into reacted and unreacted particulate systems. These results are consonant with the suggestion of Hastings *et al.* (*3*) that the particulate system is the physiological one and that the soluble system is either a precursor pool or is an artifact of the extraction procedure.

Cells were grown at 25°C in 1-liter quantities in 2.8-liter Fernbach flasks. The photoperiod was 12 hours of light followed by 12 hours of darkness (LD 12 : 12), with illumination of 8800 lu/m$^2$ (2200 lu/m$^2$ for *P. lunula*) from cool-white fluorescent lamps. *Gonyaulax polyedra* and *P. lunula* were grown in f/2-1 media (*4*) and *P. bahamense* in soil extract media (*5*). Cells were harvested from cultures with 3,000 to 10,000 cells per milliliter for preparation of extracts.

The particulate system was extracted by the procedures of others (*6*). The pellet prepared by centrifugation at 31,000*g* for 10 minutes was washed repeatedly and resuspended in tris(hydroxymethyl)aminomethane maleate (tris-maleate) buffer (0.05*M*, *p*H 8.2) containing 0.1*M* sucrose and 0.02*M* ethylenediaminetetraacetic acid; this preparation was used in these experiments. Luciferin and luciferase were prepared as described (*1*, *7*). Luciferin was partially purified on a Brown diethylaminoethyl cellulose column. Luciferase was partially purified by precipitation with ammonium sulfate (30 to 70 percent saturation) and chromatography on Sephadex G-100 in 0.05*M* phosphate buffer, *p*H 6.7.

Intensities and total light emissions were measured in absolute units of photons per second and photons (*8*). Soluble bioluminescence was assayed by adding a sample to a solution of 0.1*M* phosphate buffer, ammonium sulfate, 0.001*M* 2-mercaptoethanol and 1 mg of bovine serum albumin; final volume was 3 ml (*9*). Luciferase was assayed by the maximum rate of light emission with a saturating amount of luciferin. Luciferin was assayed by measuring the total light emitted with exogenous luciferase added to increase sensitivity. Particulate bioluminescence was assayed by injection of 0.1 ml of a sample into 1.9 ml of citrate-phosphate buffer (final *p*H 5.7, the optimum for all three species).

Release of soluble activity from the particulate system was accomplished by freezing the suspension in liquid $N_2$ and thawing it in ice water or by suspending the pellet in buffer of low ionic strength (LIS), 0.001*M* tris-maleate at *p*H 8.2. Remaining particulate activity was separated from released soluble activity by centrifuging at 31,000*g* for 10 minutes. Results are summarized in Table 1. For *P. bahamense* and *P. lunula*, the particulate system showed greater activity in the soluble assay for luciferin than in the particulate assay. There was also a greater recovery of luciferin in the LIS supernatant than the loss measured from the particulate system. For *P. lunula*, an appreciable amount of luciferin was recovered in the control supernatant, even though centrifugation and resuspension alone did not significantly decrease particulate activity. These results may indicate nonspecific binding of luciferin to the active particles or to contaminating particles, or may indicate specific binding to the active particles at sites where luciferase is absent or inactive. It is also possible that the bioluminescent quantum yield is higher in the soluble reaction than in the particulate. Latent activity might explain the large differences between the soluble luciferase values for LIS supernatants and controls (Table 1).

The particulate systems, when assayed by the soluble method for both luciferin and luciferase, always gave significant amounts of light. The minimum of four cycles of centrifugation and resuspension should have removed all truly soluble unbound substances. Therefore, the soluble activity might be due to the particulate system itself reacting at suboptimal conditions. Alternatively, luciferin and luciferase may dissociate from the particles in the high salt concentration in the assay, or the added soluble luciferin or luciferase component may react with the particle-bound luciferase or luciferin, respectively. We have been able to eliminate particulate system activity for all three species by incubation under conditions for the soluble assay.

Luciferin was incorporated into the particulate system of all three species. For *P. lunula* and *P. bahamense* addition of exogenous luciferin to a freshly extracted particulate system from these species always resulted in a much higher

Table 1. Solubilization of the particulate systems. The control (C) is the particulate system centrifuged and resuspended the same number of times as the particulate system treated with buffer of low ionic strengths (LIS), 0.001*M* tris-maleate, or frozen and thawed (FT). The particulate system of *P. bahamense* was treated with exogenous luciferin, any that remained unbound being removed by two cycles of centrifugation and resuspension. Results without luciferin addition were qualitatively the same (spnt, supernatant).

| Preparation | Particulate assay ($10^9$ photons) | Soluble assay | |
|---|---|---|---|
| | | Luciferin ($10^9$ photons) | Luciferase ($10^9$ photon/sec) |
| | *Gonyaulax polyedra* | | |
| C | 108 | 23 | 28 |
| C spnt | <1 | 7 | 6 |
| LIS | <1 | <1 | 8 |
| LIS spnt | <1 | 55 | 88 |
| FT | 64 | 10 | 12 |
| FT spnt | 1 | 36 | 36 |
| | *Pyrodinium bahamense* | | |
| C | 310 | 819 | 123 |
| C spnt | <1 | 64 | 25 |
| LIS | 110 | 466 | 141 |
| LIS spnt | <1 | 410 | 116 |
| | *Pyrocystis lunula* | | |
| C | 25 | 71 | 11 |
| C spnt | 0.8 | 41 | 8 |
| LIS | 6 | 27 | 5 |
| LIS spnt | 0.3 | 81 | 55 |

Table 2. Stepwise treatment of the particulate system, assayed by the particulate method. For all species the first-order initial decay rate was extrapolated to obtain the total light emission. *Pyrocystis lunula* alone showed a change in the initial decay rate in the presence of soluble luciferin, as well as a slow reaction that made the total light emission even larger than that reported here by approximately a factor of 10. After unbound luciferin was removed, the decay rate was the same as that of the starting material. Relative light emission is expressed as the percentage of activity at step 3 (CR, centrifuged and resuspended).

| Extraction step | *Gonyaulax polyedra* | | *Pyrocystis lunula* | | *Pyrodinium bahamense* | |
|---|---|---|---|---|---|---|
| | $10^9$ photons | Percentage | $10^7$ photons | Percentage | $10^9$ photons | Percentage |
| 1. Freshly extracted | 135 | | 162 | | 1.1 | |
| 2. Luciferin added | 279 | | 783 | | 880 | |
| 3. CR | 119 | 100 | 352 | 100 | 505 | 100 |
| 4. Acid reacted; CR | <0.04 | <0.04 | 0.4 | 0.1 | 0.3 | <0.1 |
| 5. Luciferin added | 4.8 | 4 | 97 | 28 | 189 | 38 |
| 6. CR | 1.2 | 1.0 | 17 | 5 | 129 | 26 |
| 7. Acid reacted; CR | <0.03 | <0.03 | 0.2 | <0.1 | 0.2 | <0.1 |
| 8. Luciferin added | 0.5 | 0.4 | 5 | 1 | 67 | 13 |

total light emission in the particulate assay (Table 2). For *P. bahamense* this increase was by a factor of several hundred. In most *G. polyedra* extracts no increase was observed, although occasionally a twofold increase could be obtained.

In all three species the addition of exogenous luciferin to a particulate system that had been stimulated by acid and resuspended at *p*H 8.2 recharged the system, so that it again emitted a flash of light when the *p*H was lowered to 5.7. This process could be repeated several times with decreasing yields. The acid-stimulated particles to which exogenous luciferin was added were centrifuged at 31,000*g* for 10 minutes and resuspended to demonstrate the binding of luciferin to the particles.

The activity of the luciferin-recharged particles in the presence of soluble luciferin was always higher than the activity of these particles after centrifugation and resuspension in buffer free of luciferin (Table 2, steps 2, 3, 5 and 6). This may be due to loss of enzyme activity or release of bound luciferin during centrifugation and resuspension, or it may be that luciferase bound to particles can react with free luciferin in the particulate assay. The effect is most marked for *P. lunula*. The recharged *P. lunula* system in the presence of soluble luciferin showed a slower rate of light emission than the same system after centrifugation and resuspension in buffer free of luciferin.

Comparison of kinetics, profiles of activity as a function of *p*H, and emission spectra for "native" and recharged particulate systems offer evidence that recharging may be a physiological event. The kinetics of the rise and decay are identical within a species. All activity profiles between *p*H 4.2 and 6.7 are roughly parabolic with half-widths of approximately 0.6 *p*H unit. The *p*H optima for all recharged and native particles are identical. There are some differences in activity at suboptimum *p*H, which are not consistent among the species. However, the mean half-width of the profiles for the reconstituted particles differs from that of the native particles by less than 12 percent.

By a modification of the technique of simultaneous measurement of the light reaction by two phototubes, each with its own interference filter (*10*), we have been able to demonstrate that the relative shapes of the emission spectra of all the particulate systems, native and recharged, are essentially identical. The bioluminescence emission spectrum of the native particulate system is the same as that of mechanically stimulated cells (*3*) and the in vivo spectra of the three species are identical (*8*).

Our experiments do not settle the question of whether in vivo bioluminescence is due to soluble or particulate components. However, the demonstration, in additional species, of particulate systems which are labile, contain dissociable luciferin and luciferase, and can be made to recycle by the addition of luciferin strengthens the argument that the particulate system is the physiological one (*11*).

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**References and Notes**

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9. Optimum *p*H and ammonium sulfate concentration are: *G. polyedra*, 6.7 and 0.7*M*; *P. bahamense*, 6.7 and 0.9*M*; *P. lunula*, 7.2 and 1.2*M*.
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11. It has come to our attention that Fogel and Hastings demonstrated that the particulate system of *G. polyedra* could be recharged with *G. polyedra* luciferin [M. Fogel, thesis, University of Illinois (197→ M. Fogel and J. W. Hastings, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 690 (1972)].
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## Abandoned Larvacean Houses: A Unique Food Source in the Pelagic Environment

Abstract. *Observations made by using conventional scuba techniques reveal that abandoned larvacean houses serve as food sources for marine, planktonic copepods. Techniques have been developed for photographing these houses in the field for the first time. The abundance of larvacean houses in the open ocean indicates that they are important in pelagic food webs and as a source of particulate organic matter.*

Larvaceans, pelagic tunicates of the class Appendicularia, are one of the commonest elements of marine zooplankton. As a major metazoan link between the nannoplankton—tiny, one-celled phytoplankters—and the larger planktonic and neritic animals of the ocean, they are central to many marine food chains. Larvaceans filter out nannoplankton through a unique feeding structure, the "house," secreted around the animal by the oikoplast epithelium of the body (Fig. 1). When the incurrent filters of the house clog with algae and detrital particles the animal abandons the house and proceeds to build a