Reports

Luminescent "Crystalline" Particles: An Organized Subcellular Bioluminescent System

Abstract. *A new type of intracellular biological particle which is functional in light emission has been discovered and isolated. As seen with the electron microscope it resembles a crystal; moreover, its high degree of birefringence suggests crystallinity. Isolated particles can be caused to emit a luminescent flash that resembles the flash emitted by the dinoflagellate,* Gonyaulax polyedra. *The particles are referred to as "scintillons."*

Although there has been cytological and kinetic evidence indicating that particulate or granular structures might be responsible for light emission in some luminescent organisms (*1*), such particles have not been previously isolated. In partially purified systems bioluminescence has been reported to occur in vitro only in soluble supernatant fractions and not in the particulate fraction (*2*). The newly discovered light-emitting particles described in this report do not emit light under the same conditions as does the soluble system (*3*). Their light-emitting potential remains relatively stable during purification; the luminescent flash is triggered by lowering the *p*H. The possibility that they may be crystalline in nature is suggested by their appearance and supported by the fact that they exhibit an unusually strong birefringence.

Evidence concerning the occurrence in vivo of these particles, which we call "scintillons," has been obtained from cytological studies of the cell with the electron microscope, which reveal structures, within the cell, that are morphologically identical to the isolated particles. Moreover the kinetics of the light emission of the isolated particles suggest that the particles are responsible for the very bright and short flash of light emitted by the living organism (Fig. 1).

These particles have been found in a photosynthetic and luminescent marine dinoflagellate, *Gonyaulax polyedra*. A typical oxygen-requiring, light-emitting system has also been isolated from the supernatant fraction of extracts from the same organism (*4*). In this latter system two organic components are required for luminescence: a heat-labile enzyme (*Gonyaulax* luciferase) which has now been purified about a hundred fold; and the relatively heat-stable substrate, *Gonyaulax* luciferin. Light production occurs when these two fractions are combined in the presence of 1*M* salt at *p*H 6.8.

The particles isolated as described here exhibit no luminescent activity when added to the light-emitting soluble system, and thereby behave as though they contain neither enzyme nor substrate. However, a very bright light emission can be obtained from the particulate fraction simply by lowering the *p*H (Fig. 1) in the presence of oxygen. No other factors have been found to be required for luminescence. When rapid mixing techniques (*5*) are used, light emission rises to a maximum intensity within about 40 milliseconds. The decline is also rapid and temperature-dependent, but the photon yield, measured as total light emitted per flash, is not temperature-dependent over the range of 3° to 23°C.

The luminescent activity of the particles during isolation, purification, and characterization has been followed with such an assay, in which a particle preparation is simply mixed with dilute acetic acid. The optimum luminescence is obtained with a final *p*H of 5.7. Maximum initial light intensity, as well as the number of photons emitted, is proportional to the number of particles present over a 100,000-fold range of dilution. The time course of the flash is thus not dependent upon particle concentration.

To obtain scintillons, cultures of *Gonyaulax* were grown under conditions of light and dark periods of 12 hours each in a supplemented seawater medium and then harvested by vacuum filtration on paper (*4*). From 750 ml of culture (5000 cells per ml) about 150 mg (wet weight) of cells were obtained. The cells were suspended in 0.1*M* sucrose buffered to *p*H 7.6 with 0.05*M* tris-maleate buffer and then broken up in a Ten-Broeck glass homogenizer. The resulting slurry was centrifuged at 1000*g* for 10 minutes in a Servall refrigerated centrifuge to remove the unbroken cells and cellular debris. The residue was discarded and the supernatant, containing the particles, centrifuged at 10,000*g* for 10 minutes to collect the active material. The particles were then resuspended in buffered sucrose and recentrifuged at 10,000*g* for 10 minutes. The washed particles were finally resuspended in either distilled water or in buffered sucrose.

Electron micrographs of these particles (photograph on cover) suspended in distilled water and then dried and shadowed on collodion-coated grids show particles that look like crystals. They are characteristically rhombohedral or twinned rhombohedrons which appear as chevrons. The length of the

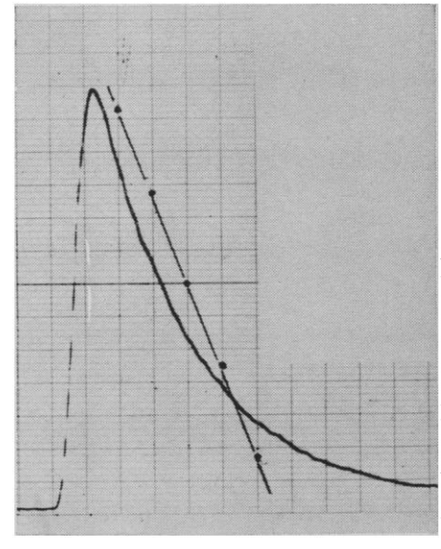

Fig. 1. Trace of the bioluminescent flash from isolated scintillons, obtained by rapidly mixing the particles with acid at 9°C. Ordinate, light intensity; abscissa, time. Mixing was complete within 4 msec. One division equals 40 msec. When carried out at a higher temperature the response is considerably faster and resembles more closely the flash of the living cell. The superimposed points are a plot of the logarithm of light intensity, illustrating that the decay of light intensity is first order.

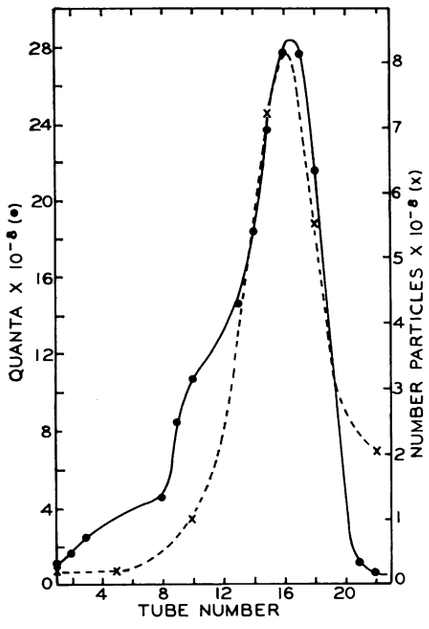

Fig. 2. Sedimentation pattern of scintillons by centrifugation in a sucrose density gradient prepared as described in the text. Bioluminescence of the fractions is correlated with the presence of scintillons. Ordinate, *left*: bioluminescence after rapid mixing with acetic acid, at 20°C, expressed in terms of the total number of quanta emitted (photon yield); *right*: number of the particles in the assay mixture. Although the number of quanta emitted per scintillon appears to be quite low, a considerable loss in activity occurred during the purification procedure; the value for the intact cell is believed to be several orders of magnitude higher.

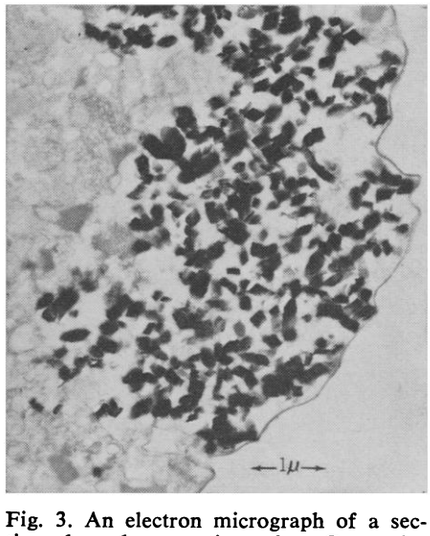

Fig. 3. An electron micrograph of a section through a portion of a *Gonyaulax polyedra* cell. The opaque structures are scintillons. Their distribution may be scattered or, as illustrated here, in clumps located primarily near the periphery of the cell. Some fine structure is evident in the sectioned crystals. A chloroplast is located at the lower left.

long axis of the particle is about 0.3 μ; however, the size ranges from 0.1 to 0.6 μ. The width and thickness are approximately equal and range from 0.1 to 0.3 μ.

For additional purification of the particles, 10 ml of the particle fraction was layered on a linear (5 to 20 percent) sucrose gradient in a 150-ml plastic centrifuge tube and centrifuged (for 2 hours at 3200 rev/min) in the swinging-bucket rotor of the model PR-1 International centrifuge. The gradient was then fractionated by puncturing the bottom of the tube with a needle and collecting drops. Samples of each fraction were subsequently assayed for light emission. The number of particles in each fraction was determined with the electron microscope by the spray droplet method of estimating particle numbers (*6*).

The quantitative relationship between luminescence in vitro and the number of particles (Fig. 2) constitutes evidence supporting the presumed light-emitting role of the scintillon. Although evidence of a more direct nature concerning this point is difficult to obtain, the high purity of the preparation strongly supports the conclusion. No alternative candidate for the light-emitting particle has been found in the electron-micrograph studies.

Figure 3 shows that scintillons occur abundantly in the cell. Although the particles are most numerous towards the periphery of the cell, they are also found in other regions. Their unusually high electron opacity is particularly striking.

Under a polarizing microscope the scintillons can be shown to possess an extremely high degree of birefringence. The coefficient of birefringence, 10^{-1}, was determined from retardation measurements in a rectified polarizing microscope with a rotating mica compensator (*7*). Because of their small size and asymetric shape, the scintillons exhibit a striking scintillation in polarized light. This is undoubtedly due to their rotatory Brownian motion. A similar scintillation attributable to the oscillatory rotation of the particles is readily visible in the living cell.

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References and Notes

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Osteoclasts: Organization in Chick Embryo Bone

Abstract. *A multinucleated endosteum was observed in the long bones of chick embryos aged 14 to 17 days. The endosteum appears to be responsible for extensive bone resorption; the bulk of the diaphyseal bone is almost completely replaced during this particular 3-day interval. Eventually, the endosteum appears to be transformed into numerous individual osteoclasts. Both the endosteum and the osteoclasts are found almost entirely on the medial surfaces of the innermost bone spicules and they seem to destroy collagen by extracellular proteolysis.*

During the course of investigations into the pathogenesis of experimental lathyrism in the chick embryo, we examined the histology of the long bones from normal embryos. Although a detailed histological analysis of bone development in the chick embryo has been reported (*1*), we observed the presence of a multinucleated embryonic endosteum which does not seem to have been described previously.

The femurs and tibias of chick embryos aged 14 to 19 days were cleaned of muscle, fixed in neutral 10-percent formalin, and decalcified in 0.2*M* ethylenediaminetetraacetic acid (EDTA) at *p*H 7.5; they were then embedded in paraffin, sectioned longitudinally at 5 μ, deparaffined, and stained with hematoxylin and eosin.