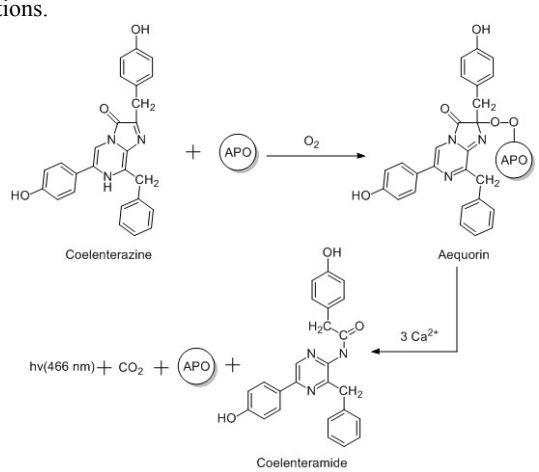
Coelenterazine

Aequorin is a calcium-sensitive bioluminescent protein from the jellyfish Aequorea victoria that has been used extensively as a microinjectable calcium indicator in cells. The aequorin complex —comprising a 22,000 MW apoaequorin protein, molecular oxygen and the lipophilic prosthetic luminophore coelenterazine —emits blue light when bound to calcium ions. Now that recombinant proteins can be targeted to specific organelles, cells and tissues,[1] recombinant apoaequorin may be even more1 useful for fine-tuning investigations of intracellular calcium.[2-3] Furthermore, coelenterazine is freely permeant to cell membranes, facilitating the reconstitution of the aequorin complex in vivo.

The luminescence of aequorin results from oxidation of coelenterazine to coelenteramide (Figure 1). If calcium ions and molecular oxygen are present, coelenterazine's luminescence response will diminish over time once it is placed in solution with either aequorin, the calcium ion–mediated decomposition product of aequorin (known as the blue fluorescent protein, BFP) or even the protein residue of BFP.[4] For this reason, solutions containing coelenterazine and any of these protein constituents should be kept as free of calcium ions as possible until the luminescence measurement is actually performed. Our Calcium Sponge™ S (BAPTA polysytrene, C-3047) is useful for removing Ca2+ from buffers. Plastic vessels are preferred over glass to avoid the leaching of Ca2+ ions from the containment vessel into stock solutions.



Scheme 1. Ca^{2+}-dependent generation of luminescence by the aequorin complex, which contains apoaequorin (APO) and coelenterazine.

Materials Storage and Handling

Coelenterazine is supplied in units of 250 µg in plastic vials. It has been the experience that coelenterazine undergoes slow oxidation in the presence of atmospheric oxygen. For this reason, the products are dried down under argon from a methanolic solution and the packaging vial is then sealed under an argon atmosphere. Upon receipt, these products should be stored desiccated at -20°C and protected from light. The packaging vial should not be opened until just prior to use and then only after it has reached ambient temperature to avoid condensation. After opening, we recommend that the packaging vial be purged with argon prior to resealing in order to protect the unused portion.

Coelenterazine can be reconstituted by dissolving in methanol or ethanol. **Do not dissolve in dimethylsulfoxide (DMSO)**; these products may be unstable in this solvent. The methanolic or ethanolic stock solutions should be stored desiccated at -20°C and protected from light; all long-term storage solutions should be as free of oxygen as possible. The molecular weight (MW) of each product is indicated on the vial label.

Coelenterazine and its analogs have extremely low solubility in water. Teranishi and Shimomura have reported[5] that 50 mM 2-hydroxypropyl-β-cyclodextrin produces a 280-fold increase in the aqueous solubility of coelenterazine, providing a way to avoid the potentially cytotoxic effects of methanol addition. Formation of aequorin in vitro from a mixture of apoaequorin (300 µg/mL in pH 7.5 buffer) and coelenterazine (7.7 µg/mL) in the presence of 0.4 mg/mL 2-hydroxypropyl-β-cyclodextrin proceeds with no adverse effects attributable to the solubilizing agent.

The concentration of the coelenterazine stock solution can be determined using the molar extinction coefficient. Generally, we dilute an aliquot of a ~1 mg/mL stock solution 1:50 with 50 mM sodium phosphate buffer, pH 7, and measure the absorbance of the diluted solution at 427 nm; the molar extinction coefficient for native coelenterazine in aqueous solution at pH 7 is 7400 $cm^{-1}M^{-1}$ at 427 nm.

References

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5. *Symp Soc Exp Biol 30*, 41 (1976).

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