Coelenterazine and Coelenterazine Derivatives

Quick Facts

Storage upon receipt:

- –20°C
- Desiccate
- Protect from light
- Avoid O_2 and Ca^{2+} exposure

Notes: Do not dissolve in DMSO

Introduction

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 more 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*.

In addition to native coelenterazine (C-2944), Molecular Probes' offers five derivatives of coelenterazine (C-14260, C-6779, C-6780, C-14261, C-6776) — designated *cp, f, h, hcp* and *n* by Shimomura and colleagues[4-6] — that confer different Ca^{2+} affinities and spectral properties to the aequorin complex (Table 1). Like native coelenterazine, these five derivatives can be used to reconstitute the aequorin complex both *in vivo* and *in vitro*.[7] Aequorin reconstituted with coelenterazine *hcp* shows very favorable characteristics, including a fast response to binding Ca^{2+} and the highest luminescence quantum yield of these five coelenterazine derivatives.[4] Aequorins containing the *cp, f* or *h* form of coelenterazine are reported to exhibit relative intensities that are 10–20 times that of apoaequorin reconstituted with native coelenterazine.[4,6] Knight and co-workers reported that aequorin reconstituted with coelenterazine *h* is more sensitive to Ca^{2+} than is the native complex, thus providing a valuable tool for measuring small changes in Ca^{2+} concentrations.[8] Coelenterazine *n* is reportedly the most useful low-sensitivity coelenterazine, producing an apoaequorin/coelenterazine complex that exhibits 10,000-fold lower luminescent intensity than the apoaequorin/coelenterazine *hcp* complex.[4]

Materials

Coelenterazine and the *cp, f, h, hcp* and *n* derivatives are supplied in units of 250 µg in plastic vials. The Coelenterazine Sampler Kit contains 25 µg samples of native coelenterazine and all five derivatives (Table 1). It has been the experience of our collaborators 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 and the derivatives 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[9] that 50 mM 2-hydroxypropyl-β-cyclodextrin produces a 280-fold increase in

Table 1. *Coelenterazines and their properties.*

Cat #	Coelenterazine Analog	Em (nm)	RLC *	Relative Intensity †	Half-Rise Time ‡ (ms)
C-2944	native	466	1.00	1	6–30
C-14260	*cp*	442	0.63	28	2–5
C-6779	*f*	472	0.80	20	6–30
C-6780	*h*	466	0.75	16	6–30
C-14261	*hcp*	445	0.65	500	2–5
C-6776	*n*	468	0.25	0.15	6–30

***** RLC = relative luminescence capacity: Total time-integrated emission of aequorin in saturating Ca^{2+} relative to native aequorin = 1.0. † Ratio of the luminescence of aequorin reconstituted with coelenterazine analog relative to native aequorin at 100 nM Ca^{2+}. ‡ Half-Rise Time: The half-rise time is the time for the luminescence signal to reach 50% of the maximum after addition of 1 mM Ca^{2+} to a standard of aequorin reconstituted with the coelenterazine analog of interest. All data are from O. Shimomura in Cell Calcium 14, 373 (1993).

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 (C-2944) in aqueous solution at pH 7 is 7400 $cm^{-1}M^{-1}$ at 427 nm.

Luminescence Generation

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.[10] 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 Ca^{2+} from buffers. Plastic vessels are preferred over glass to avoid the leaching of Ca^{2+} ions from the containment vessel into stock solutions.

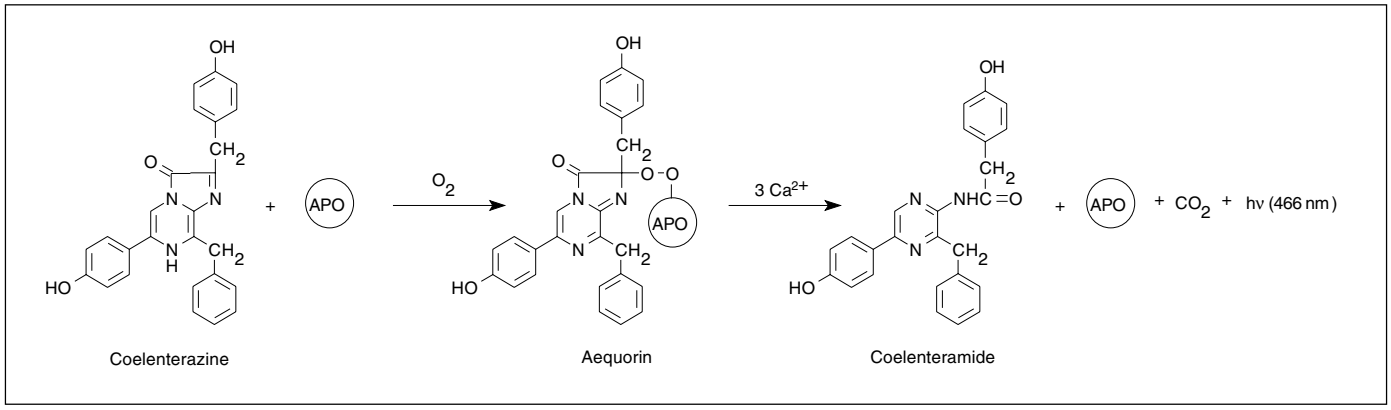

Figure 1. *Ca^{2+}-dependent generation of luminescence by the aequorin complex, which contains apoaequorin (APO) and coelenterazine.*

References

1. Methods Cell Biol 40, 339 (1994); **2.** Methods Cell Biol 40, 305 (1994); **3.** *Cellular Calcium: A Practical Approach*, J.G. McCormack and P.H. Cobbold, Eds., Oxford University Press (1991), pp 55–81; **4.** Cell Calcium 14, 373 (1993); **5.** Tetrahedron Lett 39, 5771 (1992); **6.** Biochem J 261, 913 (1989); **7.** Biochem J 296, 549 (1993); **8.** J Cell Biol 121, 83 (1993); **9.** Biosci Biotechnol Biochem 61, 1219 (1997); **10.** Symp Soc Exp Biol 30, 41 (1976).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
C-3047	Calcium Sponge™ S (BAPTA polystyrene)	1 g
C-2944	coelenterazine	250 µg
C-14260	coelenterazine cp	250 µg
C-6779	coelenterazine f	250 µg
C-6780	coelenterazine h	250 µg
C-14261	coelenterazine hcp	250 µg
C-6776	coelenterazine n	250 µg
C-6777	Coelenterazine Sampler Kit *coelenterazine and five analogs, 25 µg each*	1 kit

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