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# Efficient Bioluminescence of Bisdeoxycoelenterazine with the Luciferase of a Deep-Sea Shrimp *Oplophorus*

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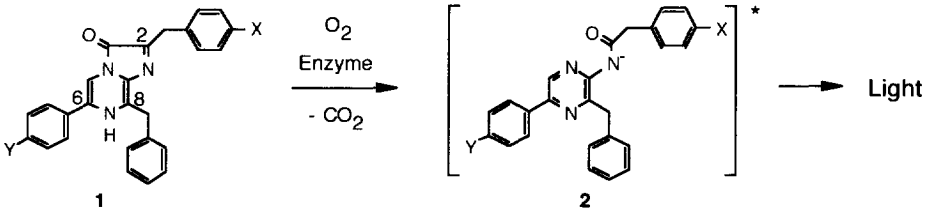
***Abstract***: The luminescence of the bisdeoxy analogue of coelenterazine (**1b** : X=Y=H) catalyzed by *Oplophorus* luciferase was highly efficient (0.19 quanta/molecule), almost comparable to the luminescence of unmodified coelenterazine (**1a** : X=Y=OH), whereas the luminescence of **1b** was very inefficient when catalyzed by apoaequorin or *Renilla* luciferase and when the aequorin regenerated with **1b** was luminesced with $Ca^{2+}$. © 1997 Elsevier Science Ltd.

Coelenterazine (**1a**, X=Y=OH) is well known as the chromogenic compound of aequorin, a $Ca^{2+}$-sensitive photoprotein from the bioluminescent jellyfish *Aequorea aequorea*[1] and it is the bioluminescent substrate (luciferin) in the luminescence of various bioluminescent marine organisms,[2] including the sea pansy *Renilla reniformis*[3] and the deep sea shrimp *Oplophorus gracilorostris*.[4] Coelenterazine contains a 3,7-dihydroimidazopyrazin-3-one ring structure and various compounds containing this structure generally show luminescence by oxidation with molecular oxygen either in the presence of enzyme (luciferase, i.e. bioluminescence) or in the absence of luciferase (chemiluminescence) in accordance to the scheme shown below. In chemiluminescence, the efficiency of the luminescence of coelenterazine analogues are low in comparison to bioluminescence (usually less than 1/10), and no characteristic substitution-effect on the efficiency was found.[3b,5] In bioluminescence, however, the substitution of the hydroxyl group on the 6-phenyl group drastically decreased the efficiency of luminescence, except when the substituent was an amino group.[6,7] Therefore, the hydroxyl group has been thought to have an important role for the efficient light emission of bioluminescence, such as molecular recognition and chemienergizing processes.




**1** **2**

In the course of our studies on the synthesis of coelenterazine analogues by using Pd-mediated cross-couplings,[8] we found that an analogue (**1b**, X=Y=H, bisdeoxycoelenterazine[9]) showed efficient luminescence with *Oplophorus* luciferase (OLase), but not with *Renilla* luciferase (RLase) or apoaequorin (Table 1).

Of the two hydroxyl groups of coelenterazine (**1a**), it has been previously known that the replacement of the hydroxyl group on the 2-benzyl group with a hydrogen atom (**1c** :X=H, Y=OH; *h*-coelenterazine[6]) does not affect neither luminescence efficiency (more than 60% activity retained) nor emission maximum.[3a,5,7] In the present study, bisdeoxycoelenterazine (**1b**), in which both the two hydroxyl groups had been substituted

**Table 1.** Bioluminescence of coelenterazine (**1a**) and bisdeoxycoelenterazine (**1b**) with *Oplophorus* luciferase (OLase), *Renilla* luciferase (RLase), and apoaequorin and of regenerated aequorin.[a]

| Enzyme | | Luminescence measured | |
|---|---|---|---|
| | | Coelenterazine (**1a**) | Bisdeoxycoelenterazine (**1b**) |
| OLase[b] | Initial rate ($x10^{10}$ quanta/s) | 190 | 150 |
| | Total light ($x10^{11}$ quanta) | 415 | 275 |
| | Emission maximum | 454 nm | 448 nm |
| RLase[c] | Initial rate ($x10^{10}$ quanta/s) | 31 | 0.10 |
| | Total light ($x10^{11}$ quanta) | 133 | 0.60 |
| | Emission maximum | 475 nm | ~400, 440 nm |
| Apoaequorin[d] | Initial rate ($x10^{10}$ quanta/s) | 0.2 | 0.003 |
| Aequorin[e] | Total light ($x10^{11}$ quanta) | 450 | 0.08 |

a) The amount of substrate used in each measurement was 0.24 nmol, except for aequorin. b) OLase (10 μg) in 3 ml of 50 mM NaCl/15 mM Tris-HCl, pH 8.3. c) Recombinant RLase (10 μg) in 3 ml of 0.1 M NaCl/25 mM Tris-HCl, pH 7.5. d) Recombinant apoaequorin (10 μg) in 3 ml of 5 mM calcium acetate/25 mM Tris-HCl, pH 7.4. e) Regenerated from 10 μg substrate and recombinant apoaequorin (0.5 mg) in 0.5 ml of 10 mM Tis-HCl/2 mM EDTA/5 mM 2-mercaptoethanol, pH 7.5 at 0 °C for overnight, then luminescence was measured by adding 10 μl of the solution to 3 ml of 10 mM calcium acetate.

with hydrogen atoms, was shown to retain most of the high luminescence efficiency of **1a** with little hypsochromic shift when tested with OLase. However, **1b** was poor substrate for RLase and apoaequorin.

According to the initial rates of light emission measured with OLase and RLase (Table 1), the ratio of the luminescence intensities of **1b** (OLase/RLase) was higher than that of **1a** by a factor of 250, indicating that bisdeoxycoelenterazine (**1b**) is greatly more specific to OLase than to RLase; in the case of an amino analogue, in which the hydroxyl group of the 6-phenyl group of **1** was replaced with $NH_2$, no significant difference in the substrate-specificity was found between OLase and RLase.[7]

The high efficiency of bisdeoxycoelenterazine (**1b**) in the presence of OLase (0.19 quanta/molecule) and the large decrease of that when RLase was used (to less than 1/400) suggested that the role of the hydroxyl group on the 6-phenyl group is probably related to the molecular recognition process of the enzymatic reaction, in accommodating the substrate molecule into the active-site cavity of an enzyme, rather than electronic effects during the chemienergizing process of luminescence reaction as previously thought. The light was emitted presumably from the excited singlet state of the amide anion (**2**) similarly to the case of **1a** with RLase.[3b]

*Oplophorus* luciferase has important advantages in its applications, such as the high stability of enzyme,[4] and the highly efficient and selective luminescence with readily available bisdeoxycoelenterazine.[9]

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9) Bisdeoxycoelenterazine (**1b**) was first reported by Hart et al.[3b] and prepared from commercially available 2-aminopyrazine by 4 steps including a cross-couplingtion of 2-amino-3-benzyl-5-bromopyrazine[8] by Stille coupling with tributylphenyltin[10] or by Suzuki coupling with commercially available phenylboronic acid and condensation with benzylglyoxal diethyl acetal.[3b]
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