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Quantum yield improvement of red-light-emitting firefly luciferin analogues for *in vivo* bioluminescence imaging

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ABSTRACT

The dimethylamino group of AkaLumine ((4*S*)-2-[(1*E*,3*E*)-4-[4-(dimethylamino)phenyl]-1,3-butadien-1-yl]-4,5-dihydro-4-thiazolecarboxylic acid), a red-light-emitting firefly luciferin analogue, was replaced by cyclic amino groups (1-pyrrolidinyl, 1-piperidino, 1-azepanyl, and 4-morpholino) to give AkaLumine analogues exhibiting desirable bioluminescence with emission maxima in the red region (656–667 nm). In particular, a bioluminescence reaction of 1-pyrrolidinyl analogue with a recombinant *Photinus pyralis* luciferase showed a higher quantum yield than that with AkaLumine, giving an improved bioluminescence intensity. The 1-pyrrolidinyl analogue also showed the strongest luminescence in whole-body luciferase-expressing mice among the analogues, indicating that a quantum yield improvement of a luciferin analogue is effective to increase bioluminescence imaging intensity.

1. Introduction

Noninvasive *in vivo* bioluminescence imaging (BLI) is now widely used for monitoring molecular and cellular events in small animals as a sensitive, rapid and inexpensive method.[1] The combination of luciferase of the North American firefly *Photinus pyralis* (*Ppy*) and its wild type substrate D-luciferin (LH_2) is most commonly used for BLI. Firefly bioluminescence (BL) is based on a luciferin-luciferase (L-L) reaction involving the enzymatic action of luciferase reacting with luciferin as a substrate.[2] That is, the luciferase catalyzes the reaction of LH_2 with ATP and oxygen in the presence of Mg^{2+} to give the light emitter, oxyluciferin (OLH), in the excited singlet state (Fig. 1A). The L-L reaction of LH_2 with *Ppy* luciferase generates yellow green light (BL maximum wavelength [λ_{bl}] = 560 nm) with a high quantum yield, which was 0.48 in a buffer at pH 8.0.[3] Because BL is based on a chemical reaction, there is no need to use an excitation light source like a fluorescence assay would. Thus, BLI provides image data with lower signal-to-noise ratio than fluorescence imaging.

The yellow green light generated by the L-L reaction of LH_2 with *Ppy* luciferase is readily absorbed by mammalian tissues containing hemoglobin and oxyhemoglobin, resulting in weakly transmitted light in BLI assays.[4] To improve tissue penetration of emitted light for BLI, it is necessary to use L-L reactions generating light in the red to near-infrared (NIR) radiation (λ = 650–900 nm) in the optical window for bioimaging. For this purpose, we and other groups have been developing luciferin analogues to emit light in the red to NIR region.[5]

Because of a high selective substrate specificity of luciferase, luciferin analogues were designed based on the LH_2 structure. As a pioneering work, it was found that aminoluciferin having the amino group instead of the hydroxyl group at the C6′ position in LH_2 shows an efficient BL with the maximum wavelength at ca. 600 nm with *Ppy* luciferase (Fig. 1B).[6] As a method to modify the aminoluciferin structure, aminoluciferin analogues conjugating a fluorescent dye at the amino group were systematically prepared. BL of the analogues conjugating BODIPY and Cy 7 (Fig. 1B), for instance, showed the λ_{bl} values at 679 and 772 nm, respectively, by intramolecular Förster resonance energy transfer.[7] As the other method, the amino group of aminoluciferin was converted to the fused azacycle, to give Cycluc series analogues ($\lambda_{bl} \leq$ 648 nm).[8]

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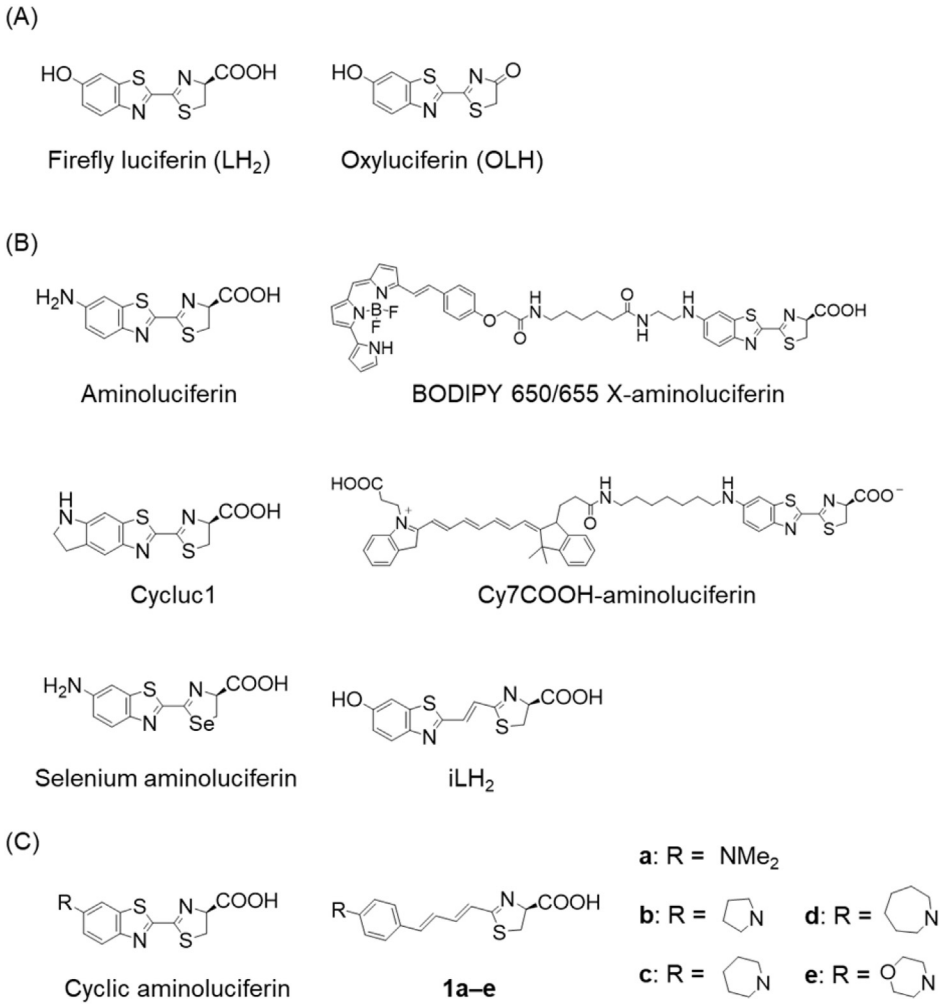

Fig. 1. Structures of (A) firefly luciferin (LH_2) and oxyluciferin (OLH), (B) previous luciferin analogues, and (C) cyclic aminoluciferin and AkaLumine (**1a**) and new luciferin analogues (**1b**–**e**).

Cycluc1 with the fused pyrrolidine (Fig. 1B), for instance, showed a high performance in BLI.[9] As a modified aminoluciferin, an analogue with a selenium atom in the thiazoline ring was also prepared, showing BL with the λ_{bl} value at 600 nm (Fig. 1B).[10]

In contrast to these methodologies, we have designed luciferin analogues by modification of the π-electronic structure of the benzothiazole moiety to the 2-phenylethenyl and 4-phenylbutadieneyl structures.[11] Because the hydroxyl and amino groups of LH_2 and aminoluciferin, respectively, work as electron-donating substituents to increase BL activities, the hydroxyl and dimethylamino groups were introduced on the phenyl group of these analogues. Among the π-modified analogues, the 4-(4-dimethylaminophenyl)butadienyl analogue **1a** ((4*S*)-2-[(1*E*,3*E*)-4-[4-(dimethylamino) phenyl]-1,3-butadien-1-yl]-4,5-dihydro-4-thiazolecarboxylic acid; Fig. 1C) showed a moderate BL activity with the longest λ_{bl} value (675 nm) using *Ppy* luciferase. This analogue and its HCl salt have been commercialized with names of AkaLumine (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and Tokeoni (Sigma-Aldrich Co. LLC., St. Louis, MO, US), respectively. Because of the red-shifted spectroscopic property of **1a**, luminescence intensities of BLI for mammals with Tokeoni were significantly stronger than those with LH_2 and Cycluc1.[12] In a similar way to modify the π-electronic structure, luciferin analogues with the ethylene and butadiene moieties inserted between the benzothiazole and thiazoline moieties of LH_2 were prepared. The ethylene-inserted analogue showed BL using mutant *Ppy* luciferase with the λ_{bl} value at 706 nm (670 nm for wild type *Ppy*; iLH_2; Fig. 1B).[13]

As a red-light-emitting luciferin analogue, AkaLumine (**1a**) is an attractive compound as a prototype to design new luciferin analogues. The molecular shape of **1a** is linear and the butadienyl moiety is flexible enough to fit the molecular space of the luciferin active site for a desirable BL activity. To improve the BL properties of **1a**, we planned to fine-tune the molecular structure of **1a** with keeping the backbone structure. As a method to fine-tune the luciferin structure, we recently reported the BL properties of aminoluciferin analogues having a cyclic amino group at C6' (Fig. 1C).[14] The cyclic amino groups have similar electron donating properties to dimethylamino group, while the molecular volumes and hydrophobicities of the cyclic amino groups are greater than those of dimethylamino group. Thus, the molecular size and hydrophobicity of luciferin analogues are successfully tuned by changing the ring size of the cyclic amino group. In fact, the analogues with the cyclic amino groups showed higher BL quantum yields (Φ_{bl}) than 6′-dimethylaminoluciferin, with the λ_{bl} values around 625 nm. In this study, we applied the fine-tuning methodology to the AkaLumine system. We prepared here AkaLumine analogues **1b**–**e** having a cyclic amino group (Fig. 1C) and investigated their BL properties.