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Synthesis and luminescence properties of biphenyl-type firefly luciferin analogs with a new, near-infrared light-emitting bioluminophore

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ABSTRACT

New firefly luciferin analogs of the 4,4′-substituted biphenyl-type were synthesized. One analog with a 4′-dimethylamino group possessed bioluminescence activity, emitting near-infrared biological window light at 675 nm suitable for deep-site bioimaging of living animals. The chemiluminescence light-emission maximum of the corresponding methyl ester of the bioluminescence active analog was 500 nm, implying that biphenyl and thiazolinone rings in the light emitter might be placed in a coplanar conformation at the polar luciferase active site.

1. Introduction

Fireflies emit light by a luciferin (substrate)–luciferase (enzyme) (L–L) reaction. The luciferase catalyzes adenylylation of firefly D-luciferin (D-LH_2) with adenosine triphosphate (ATP) in the presence of Mg^{2+}, and the subsequent oxygenation of the resulting luciferyl AMP gives oxyluciferin and yellow-green light with high efficiency (Φ_{BL}=41%) (Fig. 1).[1–4] Because the firefly bioluminescence system provides an extremely high signal-to-noise ratio, and exhibits excellent substrate specificity and sensitivity, this system is widely used in ATP detection, reporter gene assays,[5] and noninvasive whole-body bioimaging.[6] Near-infrared (NIR) biological-window light (650–900 nm) is expected to be useful for deep-site imaging, because of its low absorption by oxygenated hemoglobin and melanin as well as its diminished scattering in animal tissues.[7] Recently, bioluminescence resonance-energy transfer (BRET) systems utilizing chemically modified firefly luciferin[8a,b] and luciferase[8c,d] were successfully developed to obtain light-emission in the NIR window region,[8a,b] and the longest wavelength recorded was 783 nm.[8d] During the past decade, we designed and synthesized structurally simple luciferin analogs for color tuning of the firefly bioluminescence system, culminating in the development of analogs with a desirable light-emission maximum in the NIR biological window region.[9]

From our study of the structure–emission wavelength relationship,[9] we found that the introduction of conjugated double bonds between the aromatic parts and the thiazoline ring was very effective in elongating the wavelengths of emission maxima, producing a wavelength shift of approximately 100 nm per conjugated double bond. However, the insertion of more than three double bonds between the aromatic moieties and the thiazoline ring reduced the stability of the luciferin analogs against air and light exposure.

Herein we disclose the synthesis and evaluation of the luminescence properties of new biphenyl-type luciferin analogs (**1a**–**c**) that are expected to be stable against air and light and to emit NIR biological window light (Fig. 2).

2. Results and discussion

2.1. Molecular design and synthesis of new luciferin analogs

Using essentially the method described in the previous report,[9] we first predicted interaction of a light emitter (**oxy-1a**) generated

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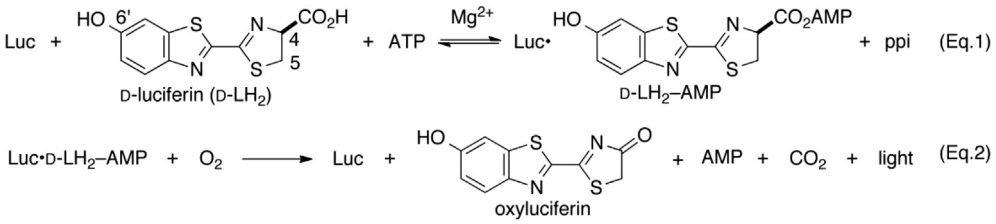

Fig. 1. Proposed reaction catalyzed by firefly luciferase. In the first step of the reaction, luciferase (Luc) catalyzes adenylylation of D-LH_2 with ATP in the presence of Mg^{2+} to generate the intermediate luciferyl-AMP (D-LH_2-AMP) accompanied with pyrophosphate (ppi) (Eq. 1). Then the oxidative decarboxylation of the intermediate gives excited-state oxyluciferin, which then releases visible light in the course of relaxation to the ground state (Eq. 2).

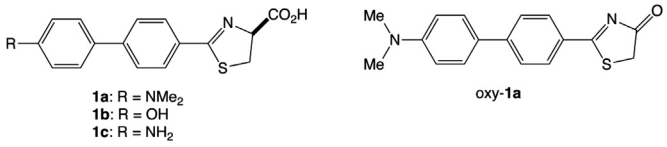

Fig. 2. Structures of new luciferin analogs (**1a**–**c**) and **oxy-1a**.

from a biphenyl analog candidate **1a** at the plausible substrate-binding site in *Photinus pyralis* luciferase. The computer-aided molecular modeling suggested that the light emitter (**oxy-1a**) might be adoptable at the active site in the luciferase (Fig. 3). Molecular modeling for predicting the plausible location of the model compound **oxy-1a** and AMP at the active site of the *P. pyralis* luciferase was performed as follows. The crystal structures of 5′-*O*-[*N*-(dehydroluciferyl)-sulfamoyl]adenosine (DLSA)-bound *P. pyralis* luciferase (PDB accession number 4G36),[10] inhibitor-bound *P. pyralis* luciferases [4E5D (2-(2-fluorophenyl)-6-methoxy-1,3-benzothiazole),[11] 3RIX (aspulvinone J-CR),[12] and 3IES (adenylylated atalurene (PTC124))[13]], and firefly oxyluciferin–AMP-bound *Luciola cruciata* luciferase (2D1R)[14] were downloaded from the RCSB Protein Data Bank. Structural alignment and construction of the figures were performed using PyMOL (DeLano Scientific; http://www.pymol.org). A DLSA-bound *P. pyralis* luciferase (4G36) was selected as a template and all other structures were superimposed onto 4G36.

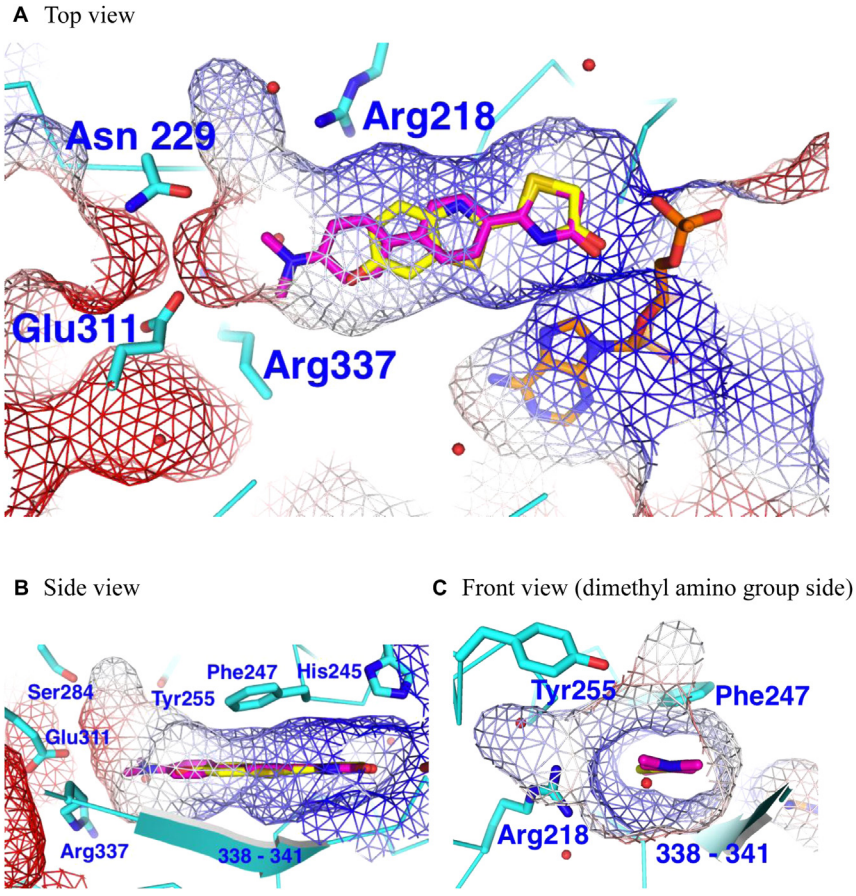

Fig. 3. Structure around a plausible ligand-binding site of *P. pyralis* luciferase and overlay model of **oxy-1a**, oxyluciferin and AMP. The backbone structure of luciferase (RCSB PDB accession number 4G36) is shown in cyan, and the electrostatic potential surface is presented as a wire mesh with negative and positive charges in red and blue, respectively. The original ligand DLSA is omitted from the figure for clarity. Side chains of Arg218, Asn229, His245, Phe247, Tyr255, Ser284, Glu311, and Arg337 are shown in stick form. Top (A), side (B), and front (C) (dimethylamino group side) views were sliced to show the binding pocket and some residues were omitted from the figure for clarity. Ribbons shown in B and C indicate the ß-strand structure of residues 338-341. Water molecules are shown as red spheres. The ligand atoms are displayed in stick form with the carbon atom of **oxy-1a** in magenta, oxyluciferin in lemon yellow and AMP in orange. Nitrogen is colored blue, oxygen is colored red, sulfur is colored yellow and phosphorus is colored deep orange.