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Synthesis of 7′-[^{123}I]iodo-D-luciferin for in vivo studies of firefly luciferase gene expression

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Abstract—D-(−)-2-(6′-Hydroxy-7′-[^{123}I]iodobenzothiazolyl)-Δ^2-thiazoline-4-caroxylic acid (7′-[^{123}I]iodo-D-luciferin) was synthesized as a novel reporter probe for in vivo studies of firefly luciferase gene expression. 7′-Iodo-D-luciferin, a nonradioactive standard, was synthesized and showed the binding property (K_M = 4.28 μM) similar to that of D-luciferin (2.53 μM) for firefly luciferase in luminescence assay.

Reporter gene is a useful concept to explore expression of the gene interested, and diverse reporter genes (β-galactosidase, β-glucuronidase, chloramphenicol acetyl transferase, alkaline phosphatase, herpes symplex virus type 1 thymidine kinase, luciferase, green fluorescent protein, and so on) have been used for a number of biological applications.[1] Imaging of gene expression has great advantages for noninvasive visualization of distribution, magnitude and duration of the gene expression, being potentially useful for diagnosis of diseases (e.g., cancer,[2] infections, and AIDS) and follow-up of gene therapy to cardiovascular disease,[3] adenosine deaminase (ADA) deficiency,[4] Duchenne muscular dystrophy,[5] etc.

Imaging modalities such as optical bioluminescent and fluorescent imaging, positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), and ultrasound have been investigated for imaging of transgene below the surface in the living subjects. Optical bioluminescent imaging is relatively inexpensive and efficient.[6] It is, however, available only for small animals and gives insufficient tomographic information. Its low sensitivity due to absorption and scattering by the organs required high dose of the reporter probe for sufficient emission. For instance, D-luciferin is needed in a dose of 126 mg/kg for firefly luciferase gene imaging.[15] MRI has high spatial resolution but requires high dose of contrast agent (>10–100 μmol/L) that leads to toxicity, cross-reactivity and other pharmacological effects.[7–10] Nuclear imaging (PET and SPECT) shows relatively low spatial resolution, however radiolabeled reporter probes are highly specific and therefore trace concentrations (pM to nM) of the probes are sufficient for good imaging.[8,10]

Herpes simplex virus type 1 thymidine kinase has been widely elucidated for noninvasive imaging of gene expression using radiolabeled reporter probes, such as 2′-deoxy-2′-fluoro-1-β-D-arabinofuranosyl-5-[^{123}I]iodo-uracil (FIAU) and 8-[^{18}F]fluoro-9-[2-hydroxy-1-(hydroxy-methyl)ethoxy]methyl]guanine (FGCV).[11–13] Firefly luciferase, which catalyzes oxidative decarboxylation of D-luciferin in the presence of ATP and oxygen, has been extensively used for the studies of gene expression. Its rapid turnover rate ($T_{1/2}$ = 3 h) and broad linear range up to 7–8 orders of magnitude enable the enzyme to emit magnified light, which allows sensitive imaging of gene expression.[14,15] Therefore, a nuclear imaging modality coupled with both firefly luciferase as a reporter gene and radiolabeled D-luciferin (Fig. 1) as a reporter probe would provide useful approaches to study gene expression in vivo.

Keywords: Gene expression; Firefly luciferase; 7′-[^{123}I]iodo-D-luciferin; Luminescence assay.
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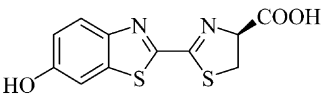


Figure 1. Structure of D-luciferin, a substrate of firefly luciferase.

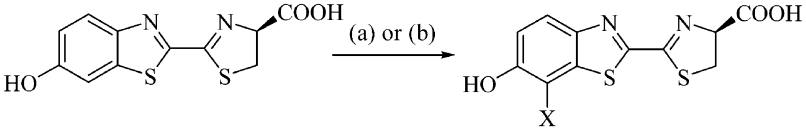
As the radiolabeling methods of D-luciferin, 6′-*O*-[^{11}C]methylation of phenolic alcohol as well as electrophilic aromatic radiohalogenation at the 7′-position was considered. *O*-[^{11}C]Methylation, however, would yield 6′-*O*-[^{11}C]methyl-D-luciferin which is known as an inhibitor of firefly luciferase.[16] This result demonstrated that the presence of the phenolic alcohol in D-luciferin may be required for a substrate of firefly luciferase. Electrophilic radiohalogenation, therefore, was used for this study. Furthermore, radiohalogen labeled D-luciferin can be applied to diverse usages, such as in vivo studies of gene expression using SPECT (^{123}I) or PET (^{124}I and ^{76}Br) and radiotherapy to specific cell transplanted with firefly luciferase gene (^{131}I).

In the synthesis of 7′-iodo-D-luciferin,[17] an unknown by-product was produced when chloramine-T was added to a solution of D-luciferin in 10% EtOH–water and NaI. To explain this side reaction, chloramine-T was added to a solution of D-luciferin in the absence of NaI and the reaction produced the same by-product based on ^{1}H NMR analysis. Therefore, D-luciferin was added to the solution containing chloramine-T and NaI to avoid direct reaction of D-luciferin with chloramine-T, which provided the desired product (Scheme 1). HPLC purification gave 7′-iodo-D-luciferin in 22% yield.

In the synthesis of 7′-[^{123}I]iodo-D-luciferin,[18] addition order of the chemicals did not make any difference in product formation. This was not surprising because radioisotope in the reaction mixture was in much lower concentration compared to NaI in nonradioactivity reaction. Low equivalent of chloramine-T (0.5 equiv) was used to avoid the side reactions (Scheme 1). The reaction was quenched by addition of water and HPLC purification (Fig. 2) gave 7′-[^{123}I]iodo-D-lucferin (t_R = 6–8 min) in 30–54% radiochemical (decay-corrected) yield and with high radiochemical purity (>99%). HPLC conditions utilizing water containing a small volume of ethanol were beneficial because ethanol is frequently used to facilitate the solubility of hydrophobic radiotracers. In this study, a 95.25:4.75 mixture of water and ethanol was used as HPLC elution solvents. Therefore, complete removal of the solvents was not necessary, which reduced the preparation time of the radiotracer.

Binding property of 7′-iodo-D-luciferin to firefly luciferase was determined using Michaelis-Menten constant (K_M) calculated from luminescence assay.[19–20] The K_M of D-luciferin was also calculated as a reference. 7′-Iodo-D-luciferin showed a similar binding property (K_M = 4.28 μM, n = 3) to D-luciferin (K_M = 2.53 μM, n = 2). It was reported that K_M of D-luciferin varied between 7.89 μM and 23 μM.[21–23] This result demonstrated that relatively bulky iodine might not cause steric hindrance for the binding of 7′-iodo-D-luciferin to the enzyme.

In conclusion, imaging of gene expression, especially exogenous gene, is a main tool to monitor gene expression repeatedly and noninvasively in biological processes. In this study, D-luciferin, a substrate of firefly luciferase, was radiolabeled for in vivo studies of gene expression. High binding property of 7′-iodo-D-luciferin suggested that this compound is also a potent substrate of firefly luciferase. Electrophilic aromatic radioiodination



Scheme 1. Reagents and conditions: (a) X = ^{127}I: NaI (3.0 equiv), chloramine-T (1.1 equiv), 18% EtOH–H_2O, rt, 10 min; (b) X = ^{123}I: Na^{123}I (150 MBq), chloramine-T (0.5 equiv), 80% EtOH–H_2O, rt, 5 min.

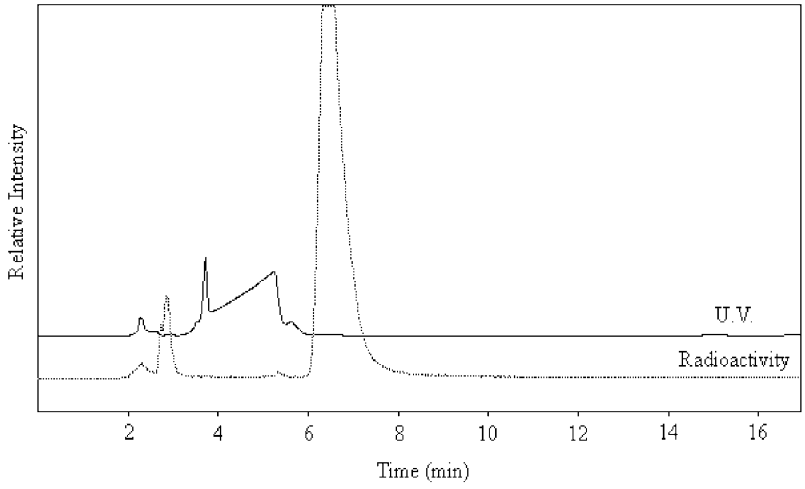


Figure 2. HPLC profile of the radioiodination mixture. D-Luciferin: t_R = 4–5.5 min (UV); 7′-[^{123}I]iodo-D-luciferin: t_R = 6–7.5 min (radioactivity).

of D-luciferin using Na[^{123}I]I and chloramine-T provided 7′-[^{123}I]iodo-D-luciferin in high radiochemical yield. Biological evaluation of this radiotracer was reported elsewhere.[24]

Acknowledgements

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17. Chloramine-T (18 mg, 80 μmol) was added to a solution of NaI (53.5 mg, 357 μmol) in 10% ethanol–water (110 mL) at rt. After the reaction solution was stirred for 30 min, D-luciferin (20 mg, 71 μmol) in 10 mL of ethanol was slowly added. The reaction mixture was stirred at rt for 10 min, and the solvents were removed in vacuo. The residue was redissolved in HPLC solvents and injected onto a reversed phase HPLC column (Alltech Econosil C18, 10 μm, 10×250 mm) eluted with a 95.25:4.75 mixture of water and ethanol at a flow 4 mL/min. Iodo-D-luciferin eluted between 6 and 7.5 min was obtained as a yellow solid in 22% yield: ^{1}H NMR (CD_3OD) δ 3.71 (2H, d, J=9.0 Hz), 5.19 (1H, t, J=9.0 Hz), 7.04 (1H, d, J=8.8 Hz), 7.83 (1H, d, J=8.8 Hz); MS (FAB) m/z 407 $(M+H)^+$; HRMS calcd for $C_{11}H_8IN_2O_3S_2$ 406.9021, found 406.9032.
18. To D-luciferin (0.36 μmol, 100 μg/100 μL in EtOH) were added Na[^{123}I]I (150 MBq) and freshly prepared chloramine-T solution (0.18 μmol, 25 μL of 1.6 mg/mL in H_2O). After stirring at rt for 5 min, the reaction was quenched with addition of H_2O (800 μL). The reaction mixture was purified by reversed phase HPLC using the same conditions described as above. The eluant was monitored simultaneously by a UV detector (254 nm) and a NaI (TI) radioactivity detector. The desired fraction eluted between 6 and 7.5 min was collected and concentrated with a gentle stream of N_2 (70–80 °C). The concentrated product (~1 mL) was diluted with saline.
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20. To an Eppendorf tube containing water (840 μL), TRIS buffer (0.5 M containing $MgSO_4$ (100 mM), EDTA (2 mM) and sodium azide (2 mM), pH 7.8), dithiothreitol (100 mM) and ATP (1 mM) were added several different concentrations of D-luciferin (9 nM–90 μM in 20% DMSO–water) or 7′-iodo-D-luciferin (0.160–160 μM in 20% DMSO–water). The incubation was initiated by adding firefly luciferase (0.5 mg/mL) to the reaction mixture. After 10 seconds, the luminescence was measured for 30 seconds by luminometer (Turner Designs). K_M was calculated using a Prism™ program.
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