Bioluminescence of 6'-aminoluciferin-analogues and beetle luciferases reveals distinct active site in Phrixotrix red-emitting luciferase and promising bioimaging applications

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Abstract

Bioluminescence color of beetle luciferase ranges from green to red with firefly D-luciferin (LH2). However some luciferin analogues can extend the range of bioluminescence colors. 6'amino-analogues have been shown to display red shifted colors with firefly luciferases, keeping a reasonable activity, and have been used as fluorescent probes for the active site of beetle luciferases. Previously, we have found that 6'-amino-substituted analogues (6'-Dimethyl-amino-luciferin, 6'-Dimethyl-amino-5-dimethyluciferin, 6'-Aminoluciferin and 6' Amino-5-dimethyluciferin) displayed red-shifted bioluminescence spectra with green emitting luciferases, and blue shifted spectra with red emitting luciferase. Now, we compared the bioluminescence spectra and activity of two novel 6'amino-substituted analogues (6'-Molpholil-LH2 and 6'-Pirrolidil-LH2) with different beetle luciferases which emit different bioluminescence colors. Similarly to the previous 6' amino-analogues, the majority of green emitting luciferases displayed red-shifted spectra with 6' Molpholil- and 6'-Pirrolidil-analogues, and reduced activity (< 1%) when compared to wild-type D-luciferin. The green emitting luciferase of *Phrixotrix vivianii* displayed similar spectra to luciferin (557 nm) with these amino-analogs (Pirrolidil-LH2: 555 nm, Molpholil-LH2: 559 nm). Noteworthy, *P. hirtus* red emitting luciferase displayed far red-shifted spectra with these amino-analogues (Molpholil-LH2: 634 nm; Pirrolidil-LH2: 644 nm), and higher bioluminescence activity (Pirrolidil-LH2: _˜15%; Molpholil-LH2: _˜10 %) when compared to other beetle luciferases. These results indicate that *P. hirtus* red emitting luciferase displays a distinctive active site, with a larger luciferin phenol binding cavity, which may accept larger 6'substituted analogues. The combination of *Phrixotrix* red-emitting luciferase with 6-substituted aminoluciferin-analogues is promising for bioimaging applications. (FAPESP 201/05426-8; CNPq 401867/2016-1)

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