Bioluminescence Spectra of Beetle Luciferases with Amino-analogues Reveals the Importance of Phenolate Group in Color Modulation

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Introduction. Beetle luciferases elicit bioluminescence of different colors, from green to red depending on the species. All of them use the same D-luciferin substrate in the light emission. However, the mechanisms and structural determinants of bioluminescence colors in different luciferases are still unknown. Many beetle luciferases were cloned and two firefly luciferases had the three-dimensional structure determined, showing the identity of the luciferin binding site. The specific interactions between the luciferase active site and oxyluciferin affecting emission spectra remain elusive. In order to understand which parts of the luciferases active site are critical, studies with luciferin analogues are very useful. Material and Methods. We synthesized two luciferin analogues - 6’-amino-luciferin and dimethyl-amino luciferin – and investigated the bioluminescence and the 2,6 TNS and tryptophan fluorescence spectra using a large set of multicolor emitting beetle luciferases cloned in our laboratories, as well as mutants involving residues critically located around the oxyluciferin phenolate. Furthermore, we calculated the $K_M$ values from the wild luciferases using the Lineweaver-Burk plotting. Results and Discussion. Almost all the green-yellow emitting luciferases display red-shifted bioluminescence spectra with amino-analogs in relation to D-luciferin, whereas Phrixotrix hirtus red-emitting and Zophobas morio protoluciferase display blue-shifted spectra. Furthermore, all luciferases displayed much higher affinity for amino-analog in relation to luciferin. The bioluminescence and fluorescence spectra of amino-luciferin show that the luciferin binding site of the red emitting luciferases, as well as the red-shifted mutants of the green emitting luciferases, are more polar than the green-yellow emitting luciferases. Conclusions. The results clearly indicate the importance of the surrounding luciferin binding site environment around the 6’ phenolate for bioluminescence color in beetle luciferases.

Word keys: amino-luciferin, bioluminescence, luciferase.

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