The active site microenvironment determines the color of emission in beetle luciferases

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Abstract

The warm glow of fireflies on a late summer evening is a cornerstone of many childhood memories. Bioluminescence is the natural process by which organisms produce light through a chemical reaction. At the heart of bioluminescence is an enzyme mediated energy transduction which oxidizes luciferin to the excited state oxyluciferin. In bioluminescent beetles, the emission color ranges from the familiar green-yellow (λmax= 540-580 nm) of most fireflies to orange and red (590-628 nm) in a few bioluminescent beetles and worms. Despite the abundance of study of the luciferase system the nature of the color tuning mechanism of beetle bioluminescence remains elusive. Herein, we describe a mutagenesis study of two far-spectrum beetle emitting luciferases to assess the contributions of several structural elements to the color of emission. Two common loop structures, loop 346-361 and loop 523-530, are responsible for binding the substrates and closing the active site of the enzyme, respectively. Mutagenesis of key residues in these structures to mimic their counterparts was sufficient to produce significant emission shifts (up to 30 nm) towards the opposite end of the spectrum. Several substitutions in each loop are responsible for determining the emission color via two competing forces: steric hindrance and electrostatic interaction; mutations in these two loop structures reveal the relative contributions of the two factors. We conclude that the electrostatic interactions of the two loops play a far greater role in determining the emission color than the steric effects of the substrate binding loop (346-361). Further energetic analysis of the wild-type and these key mutant enzymes demonstrate that the microenvironment of the enzyme active site is a major determinant of the color of bioluminescence.

Keywords: Beetle luciferase, mutagenesis, active, site, color tuning mechanism

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