Structural and Biochemical Insight into the Origin of the Green and Red Emission of Beetle Luciferases

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Abstract

The different colors of light emitted by bioluminescent beetles ranging from yellow-green to red are related to slightly different enzymes (luciferases) that catalyze the same two-stage chemical reaction-conversion of luciferin to oxyluciferin in presence of ATP and oxygen. The luciferases with known crystal structures emit in the green region of the visible spectrum. Several mutations, however, result in emission of red light (ca. 610 nm), which is close to but does not match the emission from some wild-type red-emitting luciferases (623 nm). To shed some light on the mechanism of the color "tuning" in beetle luciferases, we determined the crystal structures of several luciferases that emit light at different wavelengths. One of the structures was found to be an oligomer. The monomeric form of the luciferase is an a/b structural fold, similar to the known luciferase structures. The active site is located between the large (N) and small (C) domains, and it opens or closes by motion of the latter. To support the emission mechanism, multiple mutations were introduced in two loops that may affect the emission color. First, the loop 346-361 is at the bottom of the active site, and was found to have an effect on the energy of the emitted light. The loop 346-361 contains few amino acid substitutions that are different in the green- and red-emitting luciferases. The green emission of the wild type enzyme was shifted from 539 nm to 580 nm. Similarly, the red emission was shifted from 623 nm to 603 nm. Another loop, 523-530, was found to red-shift the emission of the green luciferase, however, it did not affect the emission of the red-emitting enzyme.

Keywords: Beetle luciferase, Crystal structure, Biochemical analysis, Mechanistic studies

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