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Structural and Biochemical Characterization of Red and Green Emitting Luciferase Enzymes

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Abstract : Bioluminescence, the emission of light from a biological process, is found in various living organisms including bacteria, fireflies, beetles, fungus and different marine organisms. Luciferase is an enzyme that catalyzes a two steps oxidation of luciferin in the presence of Mg2+ and ATP to produce oxyluciferin and releases energy in the form of light. The luciferase assay is used in biological research and clinical applications for in vivo imaging, cell proliferation, and protein folding and secretion analysis. The luciferase enzyme consists of two domains, a large N-terminal domain (1-436 residues) that is connected to a small C-terminal domain (440-544) by a flexible loop that functions as a hinge for opening and closing the active site. The two domains are separated by a large cleft housing the active site that closes after binding the substrates, luciferin and ATP. Even though all insect luciferases catalyze the same chemical reaction and share 50% to 90% sequence homology and high structural similarity, they emit light of different colors from green at 560nm to red at 640 nm. Currently, the majority of the structural and biochemical studies have been conducted on green-emitting firefly luciferases. To address the color emission mechanism, we expressed and purified two luciferase enzymes with blue-shifted green and red emission from indigenous Brazilian species Amydetes fanestratus and Phrixothrix, respectively. The two enzymes naturally emit light of different colors and they are an excellent system to study the color-emission mechanism of luciferases, as the current proposed mechanisms are based on mutagenesis studies. Using a vapor-diffusion method and a high-throughput approach, we crystallized and solved the crystal structure of both enzymes, at 1.7 Å and 3.1 Å resolution respectively, using X-ray crystallography. The free enzyme adopted two open conformations in the crystallographic unit cell that are different from the previously characterized firefly luciferase. The blue-shifted green luciferase crystalized as a monomer similar to other luciferases reported in literature, while the red luciferases crystalized as an octamer and was also purified as an octomer in solution. The octomer conformation is the first of its kind for any insect's luciferase, which might be relate to the red color emission. Structurally designed mutations confirmed the importance of the transition between the open and close conformations in the fine-tuning of the color and the characterization of other interesting mutants is underway.

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