Residues affecting the ATP binding site of *Phrixotrix* railroadworm red emitting luciferase

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INTRODUCTION Luciferases are enzymes responsible for bioluminescence. These enzymes catalyze the highly exergonic oxidation of substrates known as luciferins, the energy being released preferentially as light with high quantum yields. *Phrixothrix hirtus* (PxRE) railroadworm luciferase, cloned and characterized by our group, is the only one that naturally emits red light, and has a high affinity for luciferin and ATP, being potentially useful for bioanalytical assays in pigmented samples. However, this enzyme has low quantum yield when compared to green emitting luciferases. Based on prior knowledge of the structure and function of this and other luciferases, we aim to develop a novel red light emitting luciferase with increased catalytic efficiency, reducing the K_M for the substrates and increasing the catalytic constant by techniques of site-directed mutagenesis. **MATERIAL AND METHODS** *P. hirtus* railroadworm luciferase cDNAs was previously subcloned into the pCold vector (Takara), expressed in *E.coli* and purified by affinity chromatography on nickel matrix. Site-directed mutagenesis was performed using a Agilent site-directed mutagenesis kit. The luminescence intensity was measured using a AB2200 luminometer (ATTO, Tokyo) in counts per second. The K_M values were calculated using Lineweaver−Burk plots taking the peak of intensity (I_0) as a measure of V_0. **RESULTS AND DISCUSSION** The two mutations performed in *Phrixothrix hirtus* railroadworm luciferase (RE H241F and RE K441A) decreased five times and half the K_M value for ATP, increasing the affinity for this substrate. The K_M value for luciferin was, however, increased. These same residues when mutated in *Photinus pyralis* luciferase resulted in both K_M for ATP and for luciferin decreases, therefore, these residues are important for the ATP-binding site in different luciferases. **CONCLUSION** The mutated residues are important for the ATP binding site. Now we are performing tests for the catalytic efficiency of these mutants and comparing with other mutated luciferases.

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