Luciferin binding site residues affecting catalytic efficiency of Phrixothrix railroadworm red emitting luciferase

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Abstract

Beetle luciferases are responsible for production of bioluminescence of different colors from green to red. Among several beetle luciferases that have been studied, *Phrixothrix hirtus* (PxRE) railroadworm luciferase is still the only one that naturally emits red light, displaying high affinity for luciferin and ATP, being potentially useful for bioanalytical assays in pigmented samples. However, this enzyme has low quantum yield (15%) when compared to green emitting luciferases (40-60%). Based on prior knowledge of the structure and function of this and other beetle luciferases, we are investigating residues that affect luciferin and ATP KM as well as bioluminescence colors, in order to develop novel red light emitting luciferase with increased catalytic efficiency and red-shifted emissions using site-directed and random mutagenesis techniques. We prepared the mutants S195T, H241F, H242K, F244Y, C311S, L334R, N351E, R353E, K441A, measured their activity, bioluminescence spectra, KM values and $k$cat. The RE mutants H241F, H242K C311S, N351E, R353E, K441A, displayed similar bioluminescence spectra, decreased the KM value for ATP, increasing the affinity for this substrate, however the KM value for luciferin for most of these mutants increased. The mutants H242K, L334R and R353E showed similar KM value for luciferin to the wild-type enzyme. Among all mutants, L334R, was the one that had the most relevant effect, causing a blue-shift, and a decrease of KM for both substrates increasing the catalytic efficiency. Considering the increased catalytic efficiency for ATP in the single mutants REH241F and RE R353E, we also prepared the double mutant H241F/R353E which, surprisingly, lost the luminescent activity. Altogether, the above results indicate that the mutations H241F, C311S, N351E, K441A, despite affecting luciferin binding, improve ATP affinity, and that position 334 modulates luciferin binding and bioluminescence spectra in *Phrixothrix* red emitting luciferase. (FAPESP 2010/05426-8; CNPq 401867/2016-1)

Keywords: Luciferin binding site, Site, directed mutagenesis

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