The proton and metal binding sites responsible for pH and metal sensitivity in firefly luciferases

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Abstract

Firefly luciferases elicit yellow-green light under physiological and alkaline conditions, however at acidic pH, higher temperatures or in the presence of divalent heavy metals the spectra changes to red, a property that has been called pH-sensitivity. Despite many decades of studies, the specific proton and metal binding sites responsible for triggering red light emission, and the mechanism of pH-sensitive color tuning have remained enigmatic. Through site-directed mutagenesis, modelling and potential calculations using 3 firefly luciferases emitting distinct bioluminescence colors (*Amydetes vivianii*; 539 nm; *Cratomorphus distinctus*: 548 nm; *Macrolampis* sp2: 569 nm), we provide compelling evidences that the side-chains of residues H310, E311, R337 and E354 constitute the proton and metal binding site responsible for metal and pH-sensitivity in firefly luciferases. The side chains of the residues H310, E311 and E354 coordinate metals such as Zn2+. Substitutions at these positions indeed modulate metal sensitivity in firefly luciferases. The carboxylate of E311 may play an essential role as a base during excited oxyluciferin phenolate proton transfer. The salt bridges between H310/E354 and especially E311/R337 keep a closed conformation of the luciferin binding site, apparently retaining the excited oxyluciferin released proton near its phenolate group into a high energy state. Protonation, metal binding or mutation of these residues weaken these electrostatic interactions, reducing the force to retain the proton near excited oxyluciferin phenolate, polarizing the environment and resulting in red light emission. (**Financial support:** FAPESP 2010/05426-8 and CNPq 401867/2016-1)

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