A New Luciferin binding site residue affecting bioluminescence colors in beetle luciferases


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Beetles luciferases emit bioluminescence of different colors, ranging from green to red, depending on the species. They are functionally classified in pH-sensitive and pH-insensitive, depending of the sensitivity of their spectrum to pH, temperature, among other factors. During the past years we have cloned several new beetle luciferases of the two classes, including the pH-insensitive Pyrearinus (\(\lambda_{\text{max}}=534\) nm) green emitting luciferase, Phrixotrix hirtus red emitting luciferase (\(\lambda_{\text{max}}=623\) nm), and more recently the pH-sensitive Macrolampis sp2 (\(\lambda_{\text{max}}=564\) nm) and Amydetes fanestratus (\(\lambda_{\text{max}}=538\)) firefly luciferases. With these models at hand, we have investigated the structural determinants of bioluminescence colors. Recently we started to investigate the luciferin binding site residues substitutions on bioluminescence colors and the residues affecting pH-sensitivity in firefly luciferases. We found that substitution at residues C/S313 in green-yellow emitting luciferases results in variable red shifts in their bioluminescence spectra, affecting the kinetic properties including luciferin \(K_M\). Modelling studies showed that this residue is located in the benzothiazolyl side of the luciferin binding site. Furthermore, we have identified an important active site stabilizing polar interaction between residue S250 and R218 which may influence pH-sensitivity and bioluminescence colors in green emitting luciferases. These results bring new insights on the structural mechanisms of bioluminescence colors in beetle luciferases.

Key-words: Beetle luciferase, site-directed mutagenesis, bioluminescence.

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