Bioluminescent spectra of native and mutant firefly luciferase as a function of pH

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Firefly luciferase *Luciola mingrelica* with point mutation His433Tyr was obtained, that has bioluminescence maximum at 606 nm whereas native enzyme has one at 560 nm. Bioluminescence spectra in the pH interval 5.6-10.2 demonstrated big difference in relative content of keto- and enol- forms of oxyluciferin for native and mutant enzymes. At pH ≥ 7.0, only enol’s bioluminescence was observed for native luciferase whereas both forms were observed for mutant one, and keto- form predominated over enol-form. Relationship between keto- and enol-forms equals to 50% at pH 6.5 for native luciferase and at pH 8.9 - for mutant one. Therefore, the pK value for equilibrium keton ↔ enol in the active site of luciferase was shifted to the higher pH after mutation His433Tyr. The mechanism that can be responsible for the pK changes observed is discussed.