Bioluminescence Spectra of Native and Mutant Firefly Luciferases as a Function of pH

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Abstract—Bioluminescence spectra of the wild-type recombinant *Luciola mingrelica* firefly luciferase and its mutant form with the His433Tyr point mutation were obtained within the pH 5.6-10.2 interval. The spectra are shown to be a superposition of the spectra of the three forms of the electronically excited reaction product oxyluciferin: ketone (λ_{max} = 618 nm), enol (λ_{max} = 587 nm), and enolate-ion (λ_{max} = 556 nm). The shift in λ_{max} by 40 nm to the red region in the mutant luciferase bioluminescence at the pH optimum of enzyme activity (pH 7.8) is explained by the change in the relative content of different oxyluciferin forms due to the shift in the ketone ↔ enol ↔ enolate equilibria. A computer model of the luciferase–oxyluciferin–AMP complex was constructed and the structure of amino acid residues participating in the equilibrium is proposed. Computer models of the protein region near the His433 residue for the wild type and mutant luciferases are also proposed. Comparison of the models shows that the His433Tyr mutation increases flexibility of the polypeptide loop that binds the N and C domains of luciferase. As a result, the flexibility of the C domain amino acid residues in the emitter microenvironment increases, and this increase may be the reason for the observed differences in the bioluminescence spectra of the native and mutant luciferases.

Key words: firefly luciferase, luciferin, ATP, bioluminescence spectra, protein structure, computer models, amino acid mutation