Interaction of oxyluciferin’s analogs, dimethyl oxyluciferin and monomethyl oxyluciferin, with firefly luciferase

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Fluorescent properties, as well as stability of oxyluciferin’ analogs, dimethyl oxyluciferin (DMOL) and monomethyl oxyluciferin (MMOL), in large pH range were studied. DMOL and MMOL were used as effectors to study dynamic properties of luciferase active site. The binding constants of DMOL and MMOL with native and mutant luciferases were determined at pH 6.0-9.0. DMOL and MMOL were shown to bind to the enzyme more effectively when its phenolic group was protonated. The short-wave shift of fluorescence maximum and the increase in fluorescence intensity were shown for DMOL and MMOL bound to luciferase. It is explained by lower polarizability, ∆f, (in comparison with water solution) of the luciferase active site.