Emission Properties of Oxyluciferin and its Derivatives in Water: Revealing the Nature of the Emissive Species in Firefly Bioluminescence

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The phenomenon of bioluminescence is a fascinating natural process by which living organisms convert chemical energy into light[1]. In the case of fireflies, the light-generating reaction involves an enzyme (luciferase) that catalyses oxidation of the substrate (luciferin)[2]. The reaction chemistry and structure of the emitter are identical for all known beetle luciferases, but the emission wavelength depends on the conditions and can vary between 536 nm and 638 nm[3]. Despite being widely used in bio-analytical assays, the chemical origin of the colour modulation remained poorly understood. In this work, we performed the first systematic steady-state and time-resolved emission study of firefly oxyluciferin and its analogues in buffered aqueous solutions[4]. Our investigations provided the individual emission spectra of all chemical forms of the emitter and the excited-state equilibrium constants in strongly polar environment with strong hydrogen bonding potential. Our data confirm the earlier hypothesis that excited-state proton transfer from the enol group is favoured over proton transfer from the phenol group. In water, the phenol-keto form is the strongest photo-acid among the isomers and its conjugated base (phenolate-keto) has the lowest emission energy (634 nm). Furthermore, for the first time we observed green emission (525 nm) from a neutral phenol-keto isomer constrained to the keto form by cyclopropyl substitution. The order of emission energies indicates that in aqueous solution, a second deprotonation at the phenol group after the enol group had dissociated (that is, deprotonation of the phenol-enolate) is not likely to occur in the first excited state. The pH-dependent emission spectra and the respective luminescence lifetimes revealed that the keto-enol tautomerism reaction, which can occur in a non-polar environment in presence of base, is not favoured in water. Although these results do not directly apply to the luciferase where the active site is considered to be of low polarity, they support the hypothesis that the excited-state potential energy surface and the related dynamics are affected by the environment of the active site. Finally, we applied several of our synthesized oxyluciferin derivatives as biosensors[5].

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References:

1. Branchini, B. R.; Southworth, T. L.; Murtiashaw, M. H.; Magyar, R. A.; Gonzalez, S. A.; Ruggiero, M. C.; Stroh, J. G. Biochemistry (2004), **43**, 7255−7262.
2. White, E. H.; Rapaport, E.; Seliger, H. H.; Hopkins, T. A. Bioorg. Chem. (1971), **1**, 92− 122.
3. Ugarova, N. N.; Brovko, L. Y. Luminescence (2002), **17**, 321−330.
4. Ghose A, Rebarz M, Maltsev OV, Hintermann L, Ruckebusch C, Fron E, Hofkens J, Mély Y, Naumov P, Sliwa M, Didier P. J Phys Chem B. (2015), **119**, 2638-2649.
5. Ghose A, Maltsev OV, Humbert N, Hintermann L, Arntz Y, Naumov P, Mély Y, Didier P. J Phys Chem B. (2017), **121**, 1566-1575.