Insights into the formation of the dioxetanone cycle in the firefly bioluminescence

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The bioluminescence process corresponds to the emission of visible light through a chemical reaction in living things. Those species can be found all around the globe from mushrooms and worms on land to jellyfish in the ocean.

In fireflies, the bioluminescence involves two molecules, a protein called luciferase and a substrate called luciferin. During the mechanistic process the luciferyl-AMP intermediate reacts with an oxygen molecule to form a high-energy intermediate (HEI), a cyclic peroxide. Although the decomposition of this HEI is well known [1], his formation is still in debate. Two different hypotheses are nowadays in competition [2]. In the first one, a basic residue removes a proton of the nearby luciferyl-AMP intermediate before the coordination of the oxygen (A). In the other one, the oxygen abstracts the proton of the intermediate (B).

Here we will present some news results on the process of formation of the HEI obtained with the help of free-energy molecular dynamics simulations and quantum mechanics/molecular mechanics (QM/MM) calculations. We have chosen to study the first hypothesis (pathway A of figure 1), with a histidine playing the role of the basic residue. We thus compute the whole pathway of the formation of the HEI, from the entrance of the oxygen in the protein to the creation of the cyclic peroxide. Both the singlet and triplet approaches are studied. In the end we gain insights of the formation of the HEI through a superoxide ion.

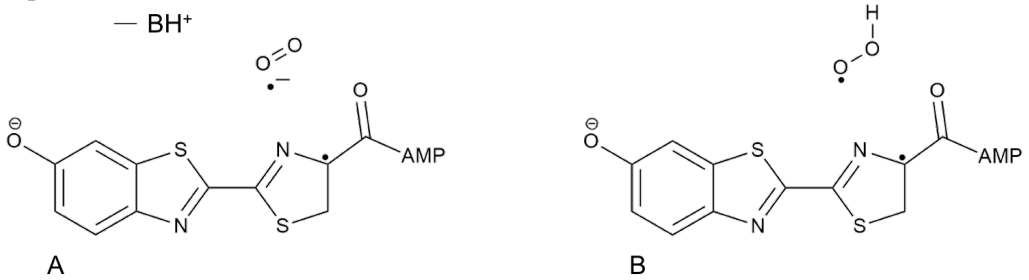

Figure 1. The two suggested pathways for the formation of the high-energy intermediate ;
A- deprotonation mechanism through a superoxide species,
B- hydrogen abstraction mechanism through a hydroperoxide species.

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[2] Sundlov, J. A.; Fontaine, D. M.; Southworth, T. L.; Branchini, B. R.; Gulick, A. M., *Biochemistry*, **51**, 6493–6495, (2012).
[3] R. Berraud-Pache and I. Navizet, *PCCP, submitted*