New insights in protonation states of the catalytic histidines in (6-4) photolyase

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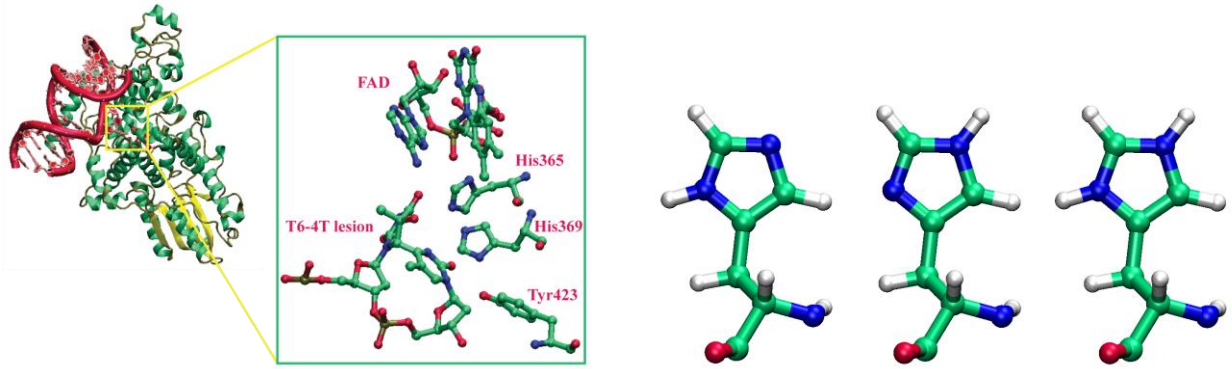

Exposure of cells to UV radiation leads to the formation of different lesions in DNA strands, one of which is a pyrimidine-pyrimidone dimer known as the (6-4) DNA lesion. (6-4) photolyases are enzymes that, together with FAD, are capable of repairing the dimer and regenerating the original monomers. Since the resolution of a (6-4) photolyase crystal structure,[1] several different mechanisms have been proposed. Nevertheless, open questions still remain.[2] It has been established that two active-site histidines and a nearby tyrosine residue are key residues in catalysis. To determine the role of the histidines, which are presumed to act as an acid-base pair, it is vital to assign their protonation states correctly. The measured hyperfine couplings of selected protons of the $FADH^{\bullet}$ radical, obtained from an EPR/ENDOR study, have been previously used as evidence in the protonation-state discussion.[3] Our QM/MM calculations of these couplings, however, suggest that they are not the most appropriate probe in this context. To further investigate the effect of the environment on the active-site histidines, their pK_a values were estimated with several approaches based on the Poisson-Boltzmann equation. Finally, a series of explicit-solvent molecular dynamics simulation were performed for each of the 9 combinations of protonation states for two adjacent histidines, with different oxidation states of the FAD cofactor. A consistent picture of the active form of the catalytic histidines emerges from a combination of the three applied methodologies.

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