The importance of a newly-discovered tetrad of tryptophan residues for the light activation of animal DNA repair enzymes (6-4) photolyases and the implications for animal photo-(magneto-)receptors cryptochromes

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Photolyases and cryptochromes are evolutionarily related flavoproteins bearing significant structural similarities but exerting diverse functions in the living organisms: photolyases are enzymes using light energy to repair UV-induced lesions in DNA, cryptochromes are photoreceptors regulating plant growth and development and participating in entrainment of circadian rhythms in both plants and animals. Cryptochromes are also believed to be responsible for the enigmatic capacity of animals to perceive the Earth's magnetic field.

A common functional element of photolyases and cryptochromes is an electron transfer chain, typically composed of three tryptophan residues, which makes it possible to reduce the flavin adenine dinucleotide (FAD) co-factor upon its photoexcitation via a reaction called "photoactivation". FAD photoreduction is supposed to be the basic process triggering signal transduction by cryptochromes. In order to be functional in DNA repair, also the photolyases need to possess the flavin cofactor in its (fully) reduced form $FADH^-$, however, it is commonly believed that the natural (dark) redox state of FAD in photolyases is already the fully reduced $FADH^-$ *in vivo* and that the photoactivation is hence not a biologically relevant process. The importance of the Trp chain for cryptochromes has also been questioned in a few recent studies.

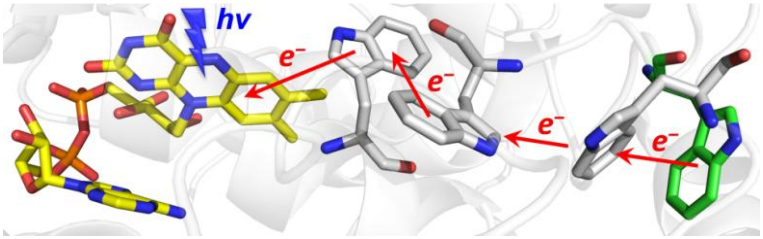

Figure 1. In addition to a usual triad, the chain of tryptophans reducing photoexcited FAD cofactor in animal (6-4) photolyases and animal cryptochromes contains an additional 4th Trp residue.

When comparing the sequences and structures of different members of the photolyase/cryptochrome superfamily, we have noticed that a sub-family of animal

cryptochromes and animal (6-4) photolyases (photolyases that selectively repair a particular lesion in the DNA, the pyrimidine (6-4) pyrimidine photoproduct) contain an additional tryptophan at a position that suggests its involvement as the fourth member of the electron transfer chain (Figure 1).

We have studied the functional role of this fourth tryptophan in an isolated (6-4) photolyase of the African clawed frog (*Xenopus laevis*) - *Xl*(6-4) *in vitro* by means of transient absorption spectroscopy. Isolated (6-4) photolyases contain a fully oxidized flavin FAD_{ox} and need to be photoactivated in order to be able to repair DNA. By comparing the first photoreduction step in the wild-type protein and in a mutant protein, in which the fourth tryptophan W370 was replaced by a phenylalanine (which cannot transfer an electron), we were able to establish that the fourth tryptophan indeed leads to stabilization of the products of photoactivation: the lifetime of the photoinduced $FAD^{\bullet-}$ $Trp^{\bullet}$ radical pair in the wild-type protein was about 4000x longer than in the mutated protein W370F, giving enough time to the present extrinsic reducing agents to reduce the $Trp^{\bullet}$ radical and thereby prevent its recombination with $FAD^{\bullet-}$ in the wild-type protein ($FAD^{\bullet-}$ is then additionally stabilized by protonation to form a neutral $FADH^{\bullet}$ radical about 200 milliseconds).[1]

Subsequent DFT calculations and MD simulations have further supported our suspicion that the 4th Trp residue is not merely stabilizing the radical on the 3rd Trp but that the 4th Trp itself is the ultimate electron donor to the excited flavin.[2]

Last but not least, we have conducted *in-vivo* experiments on transgenic *E. coli* bacteria unable to express their native cyclobutane-pyrimidine dimer (CPD) photolyase but expressing the wild-type *Xl*(6-4) photolyase or its W370F mutant.[3] After UV-irradiation of the bacteria (causing a damage to DNA) followed by their "reactivation" by visible light, the bacteria producing the wild-type *Xl*(6-4) exhibited a much higher survival rate than the bacteria expressing the W370F mutant.

We have hence shown that the dark state of FAD in (6-4) photolyases is not $FADH^{-}$ and these proteins therefore need to be photoactivated for DNA repair not only *in vitro* but also *in vivo*. Furthermore, our results have clearly shown that the 4th Trp residue and the whole tryptophan chain are vital for the photoactivation process. Possible consequences of the extended Trp chain for animal magnetoreception will be discussed.

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References:

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