

Functional dynamics of two photolyase mutants revealed by ultrafast spectroscopy

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Photolyase and cryptochrome are members of the same photoactive flavoprotein family having highly homologue structures with very different functions. A major difference between photolyases and cryptochromes is the redox state of the flavin cofactor as for photorepair the flavin should be in its fully reduced state while in cryptochromes the flavin is in oxidized state. We performed site directed mutagenesis in *E. coli* photolyase and exchanged the crucial asparagine to an aspartic acid (N378D) and investigated the changes in the photocycle imposed by the alteration of the redox state of the flavin. Ultrafast spectroscopy measurements has shown that the N378D photolyase mutant behaves like a cryptochrome, showing a photocycle very similar known in plant cryptochromes. We also constructed a second mutant photolyase in which the asparagine was altered to a cysteine (N378C) owing to the fact that in the animal cryptochromes mainly cysteine is located in the given position. Regarding the behaviour of the cysteine containing mutant we found that the flavin acquired and remained in a more stable oxidized state compared to the N378D mutant. We might conclude that the alteration of the asparagine to either an aspartate or a cysteine resulted in the change of the redox state of the flavin to the oxidized form that is characteristic of cryptochromes. This oxidized state endured longer in the N378C mutant than in the N378D.

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