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Light-induced activation of class II cyclobutane pyrimidine dimer photolyases

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ABSTRACT

Light-induced activation of class II cyclobutane pyrimidine dimer (CPD) photolyases of *Arabidopsis thaliana* and *Oryza sativa* has been examined by UV/Vis and pulsed Davies-type electron-nuclear double resonance (ENDOR) spectroscopy, and the results compared with structure-known class I enzymes, CPD photolyase and (6–4) photolyase. By ENDOR spectroscopy, the local environment of the flavin adenine dinucleotide (FAD) cofactor is probed by virtue of proton hyperfine couplings that report on the electron-spin density at the positions of magnetic nuclei. Despite the amino-acid sequence dissimilarity as compared to class I enzymes, the results indicate similar binding motifs for FAD in the class II photolyases. Furthermore, the photoreduction kinetics starting from the FAD cofactor in the fully oxidized redox state, FAD^{ox}, have been probed by UV/Vis spectroscopy. In *Escherichia coli* (class I) CPD photolyase, light-induced generation of $FADH^{\bullet}$ from FAD^{ox}, and subsequently $FADH^{-}$ from $FADH^{\bullet}$, proceeds in a step-wise fashion via a chain of tryptophan residues. These tryptophans are well conserved among the sequences and within all known structures of class I photolyases, but completely lacking from the equivalent positions of class II photolyase sequences. Nevertheless, class II photolyases show photoreduction kinetics similar to those of the class I enzymes. We propose that a different, but also effective, electron-transfer cascade is conserved among the class II photolyases. The existence of such electron transfer pathways is supported by the observation that the catalytically active fully reduced flavin state obtained by photoreduction is maintained even under oxidative conditions in all three classes of enzymes studied in this contribution.

1. Introduction

Ultraviolet light ($\lambda \leq 300$ nm) damages DNA by formation of cyclobutane pyrimidine dimers (CPDs) and (6–4) photoproducts from adjacent pyrimidine bases on the same DNA strand [1]. Such dimers are split by the action of two photoactive ($300 < \lambda < 500$ nm) damage-specific DNA-repair enzymes, named CPD photolyase [2–4] and (6–4) photolyase [5,6]. Both proteins share a common cofactor, flavin adenine dinucleotide (FAD) [7–10]. Whereas CPD photolyases specifically bind and repair CPD lesions in DNA, (6–4) photolyases reverse the (6–4) photoproduct damage. CPD photolyases are categorized into two subclasses, class I and class II, based on their amino-acid sequence similarity [11–13]. Class II CPD photolyases are markedly different from class I CPD photolyases in their amino-acid sequences, although both bind and repair the same type of DNA lesion. Therefore, it has been suggested that proteins from class I and class II form separate and only distantly related groups of enzymes, which have diverged at an early state in their evolution [12]. Class I CPD photolyases are found in many microbial organisms, whereas class II CPD photolyases have been mostly identified in higher organisms including animals [11,14,15] and plants [16–20], but also in archaebacteria [11], eubacteria [21], and single-celled algae [22]. Despite different substrate-binding specificities, class I CPD photolyases show higher similarity to (6–4) photolyases than to class II CPD photolyases [6,23,24]. Crystallographic studies on class I CPD photolyases [25–28] and (6–4)

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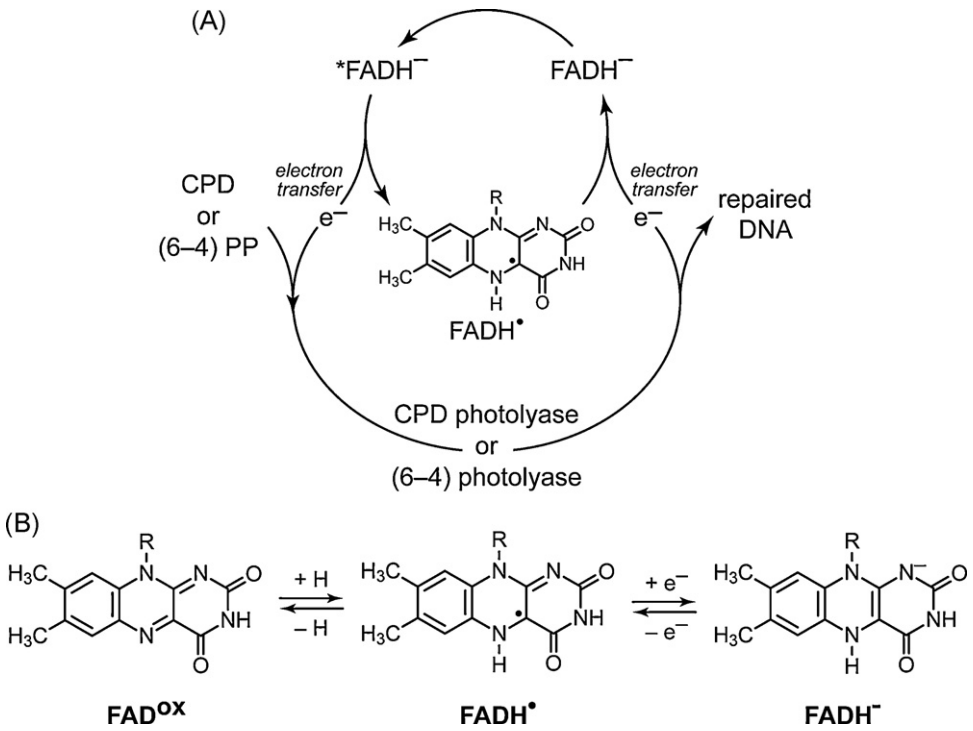

Fig. 1. (A) Proposed enzymatic repair mechanism of UV-damaged DNA (containing either CPD or (6–4) photoproduct lesions) by photolyases. (B) The different redox states of the 7,8-dimethyl isoalloxazine moiety of FAD.

photolyases [29,30] have revealed that both enzymes share well-conserved FAD binding motifs. The class II photolyases, however, are expected to differ more in FAD binding and active sites of DNA repair, based upon their greater sequence dissimilarity from other photolyases. To uncover details of substrate recognition and FAD usage, a crystal structure of a class II photolyase is highly awaited.

From studies of class I CPD photolyases, it has been proposed that the initial step in enzymatic DNA repair is a photo-induced single electron transfer from the fully reduced FAD [10,31] ($FADH^-$) to the CPD, resulting in the formation of a CPD anion radical and a neutral FAD radical, $FADH^\bullet$. The cyclobutane ring of the putative CPD radical then splits, and subsequently the electron is believed to be transferred back to the $FADH^\bullet$ radical, thus restoring the initial redox states. Hence, the entire process would represent a catalytic cycle with net-zero exchanged electrons. Panel A of Fig. 1 illustrates a simplified version of the putative DNA-repair mechanism based on results from studies of class I CPD photolyase. This mechanism is supported by recent spectroscopic examinations in which transiently formed intermediates were observed [32,33], and it was considered to apply it to class II enzymes as well.

If the enzyme is found with its FAD cofactor in an inactive oxidation state, either semi-reduced as neutral radical, $FADH^\bullet$, or fully oxidized [34], FAD^{ox}, class I photolyases and (6–4) photolyases can both undergo reversible intra-protein electron transfer reactions with the participation of amino-acid residues to reduce the flavin cofactor to its $FADH^-$ form, see panel B of Fig. 1. While this photoactivation process (also called FAD cofactor photoreduction) seems not to be of significance *in vivo* in (class I-type) CPD photolyase mediated DNA repair in *Escherichia coli* [35], it has nevertheless recently attracted much experimental [34–42] and theoretical [43,44] interest, because radical pairs generated by photoreduction of FAD^{ox} in the photolyase-related cryptochrome photoreceptors have been proposed to play a critical role in a potential magnetoreceptor function as it may be used, e.g., by migratory birds that orient themselves in the earth's magnetic field [45,46]. In *E. coli* class I CPD photolyase (*Ec*CPDI), cofactor photoreduction proceeds on a picosecond time scale [42] via a chain of tryptophan residues (W306–W359–W382 [25]) from the protein surface to its core. W306 is the terminal electron donor on the protein surface that is re-reduced on a millisecond time scale either by back electron transfer from the reduced FAD or by exogenous reductants [47]. The chain of tryptophan residues responsible for photoreduction in *Ec*CPDI is highly conserved among class I-type photolyases as well as in the related cryptochromes [12]. However, the process is poorly understood for the class II CPD photolyase, which, based on an amino-acid sequence alignment, seems to completely lack this tryptophan triad (see below). It is still unknown if electron transfer can be photo-induced in the class II enzyme in order to reduce FAD^{ox} or $FADH^\bullet$ to the catalytically active $FADH^-$ state. If so, it is of high importance to know how electrons flow from the exogenous reductant to the FAD, which is supposed to be deeply buried in the center of the protein.

In this contribution, we report (i) on studies of the photoreduction of the FAD cofactor of class II photolyases derived from *Oryza sativa* (*Os*CPDII) and *Arabidopsis thaliana* (*At*CPDII) using optical spectroscopy, and (ii) on studies of the stable neutral radicals $FADH^\bullet$ by Davies-type pulsed electron-nuclear double resonance (ENDOR). The results are compared with those from (class I) *Ec*CPDI [48] and (6–4) photolyase of *Xenopus laevis*, *Xl*6,4 [49]. The main emphasis of this study is to obtain information on intra-protein electron transfer in class II photolyases by observing the photoreduction kinetics and by probing the immediate environment of the FAD cofactor. To rationalize our findings, we propose a new electron-transfer pathway for photoreduction of class II CPD photolyases.

2. Materials and methods

2.1. Protein expression and purification

GST-tagged *O. sativa* class II CPD photolyase was overproduced in *E. coli*, and the tag-cleaved enzyme was purified as described