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Review article

Photolyase: Dynamics and electron-transfer mechanisms of DNA repair

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ABSTRACT

Photolyase, a flavoenzyme containing flavin adenine dinucleotide (FAD) molecule as a catalytic cofactor, repairs UV-induced DNA damage of cyclobutane pyrimidine dimer (CPD) and pyrimidine-pyrimidone (6-4) photoproduct using blue light. The FAD cofactor, conserved in the whole protein superfamily of photolyase/cryptochromes, adopts a unique folded configuration at the active site that plays a critical functional role in DNA repair. Here, we review our comprehensive characterization of the dynamics of flavin cofactor and its repair photocycles by different classes of photolyases on the most fundamental level. Using femtosecond spectroscopy and molecular biology, significant advances have recently been made to map out the entire dynamical evolution and determine actual timescales of all the catalytic processes in photolyases. The repair of CPD reveals seven electron-transfer (ET) reactions among ten elementary steps by a cyclic ET radical mechanism through bifurcating ET pathways, a direct tunneling route mediated by the intervening adenine and a two-step hopping path bridged by the intermediate adenine from the cofactor to damaged DNA, through the conserved folded flavin at the active site. The unified, bifurcated ET mechanism elucidates the molecular origin of various repair quantum yields of different photolyases from three life kingdoms. For 6-4 photoproduct repair, a similar cyclic ET mechanism operates and a new cyclic proton transfer with a conserved histidine residue at the active site of (6-4) photolyases is revealed.

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1. Introduction

The photolyase/cryptochrome superfamily is a diversified class

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of flavoproteins containing a non-covalently bound flavin adenine dinucleotide (FAD) molecule as the key cofactor [1,2]. The superfamily contains eight major clades (Fig. 1A) [3]. Despite their remarkably high similarity of sequence and structure, photolyases (PLs) and cryptochromes (CRYs) perform different biological functions: photolyases repair UV-induced DNA lesions (Fig. 1B and C) [4,5]; cryptochromes, being present mostly in higher plants and animals, have lost the DNA repair activity in evolution and act as blue-light photoreceptors to regulate various growth and adaptive functions [6–11].

The ultraviolet (UV) irradiation in sunlight can cause damages of DNA by inducing formation of a cyclobutane pyrimidine dimer (CPD, ~80%) and a less-frequently pyrimidine-pyrimidone (6-4) photoproduct (6-4PP, ~20%) (Fig. 2A) [2]. In CPD, two adjacent thymine bases in the same DNA strand covalently connect to form a cyclobutane ring; in 6-4PP, a complicated chemical structure is formed, where the oxygen and hydrogen atoms in one base migrate to another base, in addition to the two bases being covalently linked. Both CPD and 6-4PP lead to mutagenesis, cell apoptosis and even to skin cancer [12–14]. Photolyase recognizes these damaged thymine dimers and restores these lesions [2,15,16] through direct absorption of blue light by FAD or through energy transfer from the excited antenna chromophore [17–19]. Two different kinds of photolyases, specifically repair CPD and 6-4PP and thus are usually classified as CPD photolyases and 6-4 photolyases, respectively, corresponding to their different substrates. The CPD and 6-4 photolyases share similar primary sequences and folding structures but a photolyase that repairs one photoproduct cannot repair another. Based on sequence analyses, CPD photolyases are highly diversified and are subdivided into three classes (I-III) [20–22] and single-stranded DNA (ssDNA) specific PLs [23,24] (Fig. 1A). Crystal structures [19,25–38] of photolyase/cryptochromes, including some enzyme-substrate complex structures of both CPD [28–30] and 6-4 [31] photolyases, have been solved. Throughout the protein family, the FAD cofactor is non-covalently incorporated at the active site in an unconventional U-shaped configuration, and its lumiflavin (Lf) moiety and adenine (Ade) moiety have a short separation distance; while in other flavoproteins, the FAD is usually in a stretched, open configuration. Both CPD and 6-4 photolyases contain a fully reduced flavin adenine dinucleotide ($FADH^-$) molecule as the active cofactor. Fig. 1B and C shows the PL-substrate complex structures of the CPD photolyase from *Anacystis nidulans* (AnPL) and 6-4 photolyase from *Drosophila melanogaster* [Dm(6-4)PL], respectively. The close-up view shows that the substrate dimer flips out of the DNA double helix and inserts into the active site of PL. In both PLs, the catalytic flavin cofactor is folded and the Ade moiety is in close proximity of both the flavin isoalloxazine ring and the substrate dimer. The unique folded motif of flavin plays a critical functional role in initial photochemistry.

The flavin cofactor, first discovered in the early 1930s, is essential and ubiquitously present throughout the biological kingdom [39–44]. In nature, the majority of flavin molecules are found in flavoproteins in the form of flavin adenine dinucleotide (FAD), or flavin mononucleotide (FMN) [45]. The flavin molecules have the basic structure of 7,8-dimethyl-10-alkylosialloxazine and are chemically versatile. They can exist in three different redox states: oxidized, one-electron reduced (semiquinone), and two-electron reduced (hydroquinone). Semiquinone and hydroquinone have pK_a values of 8.3 and 6.7, respectively [46], and can be present in their neutral or anionic forms under physiological conditions. Due to their unique ability to participate in both one- and two-electron transfer processes, flavins are often involved in intermolecular electron transfer (ET) reactions, and flavoproteins are ubiquitous in biological systems and participate in a broad spectrum of key biological processes that rely on enzyme-mediated oxido-reduction reactions [42–44,47–50]. Among the five redox forms, two redox pairs, oxidized flavin/anionic semiquinone ($FAD/FAD^{\bullet-}$) and neutral semiquinone/anionic hydroquinone ($FADH^{\bullet}/FADH^-$), exist in photolyase/cryptochromes. The four states are convertible under physiological conditions through intraprotein ET and proton transfer (PT) [46] and can be spectrally differentiated by their distinct UV-vis absorptions (Fig. 2B). The steady-state spectroscopic properties of these redox flavins have been extensively studied, especially their absorption spectra in different proteins and solution [40,46,51–54].

For both CPD and 6-4 PLs, $FADH^-$ is the only catalytic state to repair DNA lesions [55,56]. The mechanism of DNA photorepair has been proposed and examined in the past thirty years [57–72]. The dynamics and mechanism of CPD repair by class I PL from *Escherichia coli* (EcPL) has been investigated with great detail in recent years; a complete photocycle was resolved and critical cyclic electron-tunneling mechanism was revealed [60–67]. For PLs from other classes, the repair activities have also been reported [24,68–71] but the overall understanding on their initial photochemistry and repair mechanisms lags behind the microbial class I members. Here we overview our recent key studies of photolyases and focus on the dynamics of the flavin cofactor and DNA repair of different CPD photolyases and the (6-4) photolyases. We have mapped out the intrinsic intramolecular ET cycles within the FAD cofactor of photolyases and subsequently, elucidated the various ET steps in DNA repair and finally unified a novel, universal ET repair mechanism for all different PLs in the superfamily [70]. A timely summary of the current field on DNA repair by photolyases was published recently by many excellent reviews in a special issue of *Photochemistry and Photobiology* dedicated to Dr. Aziz Sancar who received the Nobel Prize in Chemistry in 2015 with Tomas Lindahl and Paul Modrich for their “Mechanistic studies of DNA repair” [73].

2. FAD in photolyase: conserved folded structure

2.1. Electron shuttling and functional state

Among the four redox states of FAD, the anionic hydroquinone $FADH^-$ is the only functional state *in vivo* to repair the UV-induced DNA lesion, CPD or 64PP, which is an unusual redox state in flavoenzymes. In principle, the substrate thymine dimer can accept one electron to be reduced or donate one electron to be oxidized and then the ionic dimer subsequently splits into two bases [74,75]. The two anionic states of flavin, anionic semiquinone $FAD^{\bullet-}$ and anionic hydroquinone $FADH^-$, could donate one electron to the substrate, and the two neutral states, oxidized FAD and neutral semiquinone $FADH^{\bullet}$ can accept one electron from the substrate. For the anionic flavin, the critical question is: why does photolyase utilizes $FADH^-$, not $FAD^{\bullet-}$ as the active state? For the two neutral states, the excited flavin can obtain one electron and get reduced on the ultrafast time scale from the neighboring aromatic residues (tryptophan or tyrosine) [76–78]. One challenging question is whether the neutral flavins can accept one electron from the substrate if the active site becomes inert by mutations of aromatic residues.

Using femtosecond (fs)-resolved spectroscopy and site-directed mutagenesis, we investigated dynamics of class I PL from *Escherichia coli* (EcPL) in four redox states. For the two anionic states, a cyclic ET between the Lf and Ade moieties of the conserved folded FAD cofactor was observed and Ade behaves as an electron acceptor (Fig. 3A). For $FAD^{\bullet-}$, the forward ET from $Lf^{\bullet-}$ to Ade takes 12 ps and the back ET from Ade^- to Lf is even faster at 2 ps Significantly, for $FADH^-$, the forward ET is slow at 2 ns, followed by ultrafast back ET at 25 ps The slow forward ET in the $FADH^-$ state is a result of unfavorable driving force (+0.04 eV) [79,80] and thus ensures the fast,

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