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Invited Review

(6-4) Photolyase: Light-dependent repair of DNA damage

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Summary. DNA photolyase represents a phenomenal class of DNA repair enzymes in that it harvests the light energy to repair DNA lesions caused by ultraviolet light. Mother Nature evolves two types of photolyases, one specific for repairing cyclobutane pyrimidine dimers and the other for pyrimidine-(6-4)-pyrimidone photoproducts. Together, these two kinds of DNA photolesions account for the majority of ultraviolet light-induced DNA lesions. So far, the basic chemical steps of the enzyme mechanism of the two classes of photolyases appear to be very similar. Therefore, it will be very intersting to uncover the determinants of the different substrate specificity between the two photolyases. In this review, we focus on the discussion of the photolyase specific for repairing pyrimidine-(6-4)-pyrimidone photoproducts mainly because the research of the cyclobutane pyrimidine dimer photolyase has recently been reviewed quite extensively.

Key words: DNA repair, Photolyase, (6-4) photoproducts

Introduction

Ultraviolet (UV) light from solar irradiation is an exogenous carcinogen because it damages cellular DNA. Of all the UV photolesions, the most common are cis-syn cyclobutane pyrimidine dimers (CPD) and pyrimidine-(6-4)-pyrimidone photoproducts (Fig. 1) (see Friedberg et al. (1995) for a review). These DNA lesions are road blocks of DNA/RNA polymerases, causing nucleotide misincorporation or completely blocking the polymerase progression during replication or transcription. Therefore, mishaps such as gene mutation or cellular lethality would occur following DNA-damaging events. The relationship of UV light caused DNA lesions to human skin cancers is evident from the studies of one of the most mutated tumor suppressor gene accompanying human cancers, i.e. p53. It has been reported that more than 50% of squamous cell carcinoma from skin cancer patients carry C→T substitution and/or CC→T double base mutations in the p53 gene (Brash et al., 1991). The fact that these mutation hot spots are mostly two adjacent pyrimidines, which are also the prime target of UV light, demonstrates the significant role played by UV photoproducts in skin cell carcinogenesis. To prevent DNA damage and keep the genetic integrity, both prokaryotes and eukaryotes have DNA repair mechanisms for self defense. Of all the DNA repair systems known, the phenomenon of photoreactivation is unique in that it harvests energy from light to conduct DNA repair. The protein mediating this light dependent DNA repair reaction is called DNA photolyase.

Up to date, two types of DNA photolyases have been discovered in a wide range of species (Sancar, 1996). They are distinguished by the different substrate specificity. The activity for repairing CPD was discovered in 1950s (Rupert et al., 1958) and the responsible gene was not cloned until 30 years later (Sancar and Rupert, 1978). Since then, extensive studies have been carried out to elucidate the mechanism of action of CPD photolyase. All known CPD photolyases contain two organic cofactors. The first cofactor is a reduced flavin adenosine dinucleotide ($FADH^-$); the second is either a tetrahydrofolate or a dezaflavin. The current consensus is that the second cofactor functions as an antenna which is elevated into an energetically excited state after absorbing long wavelength UV light (i.e., blue light). This excited antenna molecule then transfers energy to $FADH^-$,which in turn donates an electron to the CPD substrate held in the enzyme active site. This negative charge induces splitting of the cyclobutane ring of CPD. Subsequently, the electron is transferred back to flavin, restoring a CPD into its original state of two neighboring pyrimidines. The climax of CPD photolyase research came in 1995 when the crystal structure of *Escherichia coli* CPD photolyase was solved at a resolution of 2.3 Å (Park et al., 1995). The three-dimensional structure of CPD photolyase shows that the protein folds into two domains, N-terminal α/ß domain and C-terminal helical domain. The flavin cofactor resides in the C-terminal domain and the folate chromophore is in the N-terminal domain. Since

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detailed discussion of CPD enzymes is available in the literature (Kim and Sancar, 1993; Sancar, 1994), this chapter will concentrate on the more recent development of a separate class of photolyase, i.e., (6-4) photolyase.

(6-4) Photolyase

The second type of photolyase, which is the focus of this chapter, specifically repairs the (6-4) photoproducts, the second most abundant UV photolesions. The classic CPD photolyase does not repair (6-4) photoproducts. Because of the structural difference of a (6-4) photoproduct from a CPD, it was thought that a simple photochemical splitting as in the case of CPD repair by CPD photolyases would be impossible for a (6-4) photoproduct (Fig. 2). It has long been thought that the (6-4) photoproducts are repaired exclusively by a light-independent repair mechanism named nucleotide excision repair. Surprisingly in 1993, Todo et al. reported for the first time that in *Drosophila melanogaster* there exists an light-dependent DNA repair activity specific for (6-4) photoproducts (Todo et al., 1993). Later on, similar activity was further extended to frogs and plants (Kim et al., 1994, 1996; Chen et al., 1994). However, as of today light-dependent repair of (6-4) photoproducts has not been detected in humans and bacteria such as *E. coli*. (Hsu et al., 1996). Currently, the cDNAs of (6-4) photolyases have been cloned from Drosophila (Todo et al., 1996) and Xenopus (Todo et al., 1997a), and candidate genes of (6-4) photolyases have also been identified in plants (Small et al., 1995; Hoffman et al., 1996), allowing the production of recombinant proteins in large quantities. From the spectroscopic studies, it is clear that the (6-4) photolyases contain a flavin cofactor. However, the nature of the second cofactor remains elusive. The fluorescence spectra of the Drosophila and Xenopus enzymes indicate the signature of a pterin like structure associated with the enzyme (S. Zhao and A. Sancar, personal communication). More structural characterizations are needed to uncover the identity of the second chromophore of (6-4) photolyases.

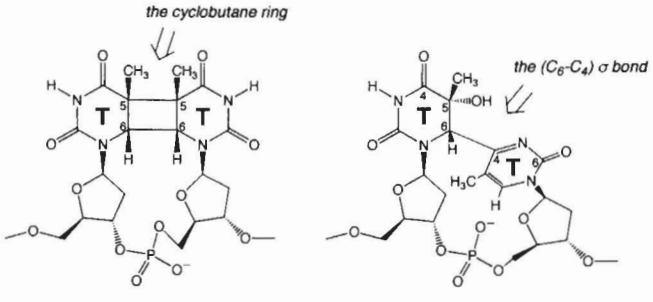

Fig. 1. Structures of the two major ultraviolet light-induced DNA lesions. For two adjacent pyrimidines (two thymidines in this figure), photolesions of a cis-syn cyclobutane dimer **(A)** or a (6-4) photoproduct **(B)** are as shown. The numbering of the pyrimidine ring is indicated.

Substrate recognition

Even though the two different types of photolyases repair strictly the particular UV photoproducts without overlapping substrate specificity, on the amino acid sequence level the two share an unexpected 40% similarity (Todo et al., 1996). Since little structural information of (6-4) photolyase is known, the implications of the three-dimentional structure of the *E. coli* CPD photolyase in CPD recognition are briefly discussed here because the (6-4) photolyase may as well use the same strategy in substrate binding. The crystal structure of the *E. coli* CPD photolyase visualizes a groove of positively charged residues laying through a stripe of the enzyme surface (Park et al., 1995). In the middle of this groove, a cavity with a size that can accomodate a CPD is observed. One side of the cavity consists of hydrophobic residues, and the other side has polar groups. This asymmetry is also a property to a CPD, in which the cyclobutane ring is hydrophobic and the opposite edges of thymines are capable of hydrogen bonding. What is even more striking is that the flavin cofactor makes up the bottom of the cavity. If indeed the cavity is occupied by a CPD, the adenine ring of the $FADH^-$ would be in van der Waals contact with the CPD, consistent with the idea that electron transfer between $FADH^-$ and CPD being important for catalysis.

In addition to the unexpected high protein sequence homology between (6-4) and CPD photolyases, the residues of the *E. coli* CPD photolyase known to be important in substrate binding are all conserved in the Drosophila (6-4) photolyase. For example, when the highly conserved Asp-399 of the Drosophila (6-4) photolyase (Asp-399 corresponds to Asp-374 of the *E. coli* CPD photolyase) is mutated into a alanine, the

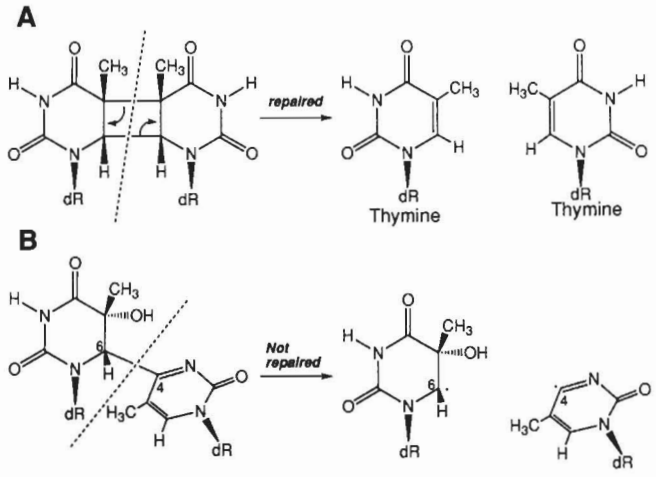

Fig. 2. Simple breakage of the covalent bond (C_6-C_4) linking the two thymines of a (6-4) photoproduct does not yield repaired products. The repair of a CPD is indicated in **(A)**. Breakage (denoted by a dotted line) of the cyclobutane ring results in two canonical thymines. In contrast, direct breakage of the (C_6-C_4) σ bond of the (6-4) photoproduct would yield damaged thymines **(B)**. dR represents deoxyribose.

binding affinity to (6-4) photoproducts is totally abolished (X. Zhao and A. Sancar, unpublished results). Given these similarities between CPD and (6-4) photolyases, it is very tempting to speculate that the (6-4) photolyase also contains a hole which fits a (6-4) photoproduct. When substrate DNA is bound by the enzyme, the (6-4) photoproduct flips out the DNA duplex and into the hole as described for the CPD photolyase. In the absence of structural data, there are several criteria that can be used to judge if base flipping is part of the substrate binding mechanism. They include: if base flipping is utilized, the targeted base would be bound by the enzyme with a higher affinity when it is in a mismatched sequence context. This is due to the lower free energy needed to flip out a mispaired base. In addition, the binding affinity for the targeted base in single-stranded DNA should not be attenuated compared with the binding affinity of the targeted base in double-stranded DNA because the flexibility of the targeted base in single-stranded DNA compensates for the binding energy provided by the second strand. Finally, base flipping would create single strandedness immediately surrounding the target base. Indeed, recent experiments by Zhao et al. (1997) showed that the Drosophila enzyme meet all three criteria. Therefore, (6-4) photolyases likely flip out the (6-4) photoproduct during substrate recognition.

Catalysis

Flavin adenine dinucleotide (FAD) works as a redox cofactor in many enzymes. As described for the *E. coli* CPD photolyase, it is the reduced FAD ($FADH^-$) that is active for repairing CPDs. Similarly, after treatment of a reducing agent such as dithionite, the repair efficiency of (6-4) photolyase was enhanced by 4-5 fold, indicating that $FADH^-$ is the active species for repair. What the dithionite treatment does is to enrich the fraction of (6-4) photolyases containing reduced FAD, hence the higher repair activity. Once again, analogous to the CPD enzyme, the electron donation from reduced FAD to the (6-4) photoproduct is very likely operating in the catalytic mechanism of (6-4) photolyases (Hitomi et al., 1997; Zhao et al., 1997). However, the nature of the photon-receiving antenna molecule in (6-4) photolyases remains mysterious.

As soon as a negative charge is received by (6-4) photoproduct from $FADH^-$, reversal of the photodimer into two canonical pyrimidines will be initiated. An important question is what intermediates are generated in the reversal reaction. Since an oxetane species is known to be intermediating the formation of a (6-4) photoproduct from a dipyrimidine, it was proposed that the (6-4) photolyases catalyze the repair of (6-4) photoproducts by pushing the "forward reaction" backward. Thus, the oxetane intermediate of the forward reaction must be intermediating the repair reaction by the enzyme. Fig. 3 illustrates the proposed repair mechanism. Consistently, in studies using model compounds the oxetane adduct of thymine and benzaldehyde or acetophenone is able to split into the original reactants in the presense of cation or anion sensitizers (Prakash and Falvey, 1995). As a different approach and due to the extreme instability of oxetane structure, oxetane intermediate analogs with a sulfur replacing oxygen were synthesized (named thietane). The thietane anolog was found to be able to revert to the normal base when irradiated with light in the absence of the (6-4) photolyases (Liu and Taylor, 1996). However, when the thietane analogs were subjected to the (6-4) photolyase, it was found that none of the thietane compounds is substrate of (6-4) photolyases. To resolve this issue of repair reaction intermediates, the employment of ultrafast techniques like the femtosecond laser spectroscopy would be helpful in directly observing the repair intermediates on a "real-time" basis.

In summary, mechanistically the (6-4) photolyase is similar to the CPD photolyase in almost every aspect; this similarity may be reflected by the protein sequence homology. Interestingly, why is one repairing CPD and the other repairing (6-4) photoproduct? Is there a hidden switch that controls the substrate specificity. Although so far no DNA photolyase activity has been detected in

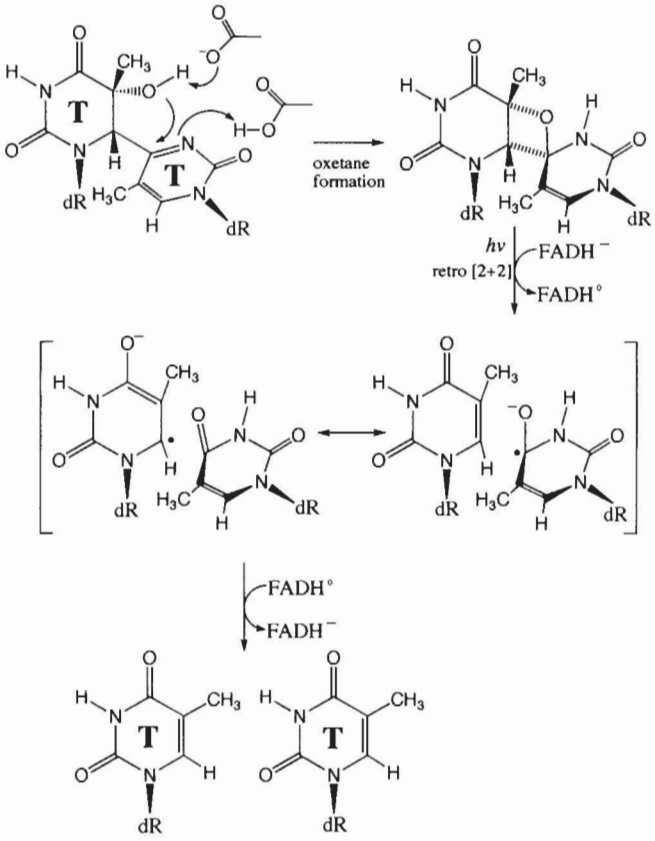

Fig. 3. Proposed mechanism of the (6-4) photolyase. Two carboxylate residues of (6-4) photolyases are shown to take part in the formation of the oxetane intermediate. This model is adapted from Kim et al. (1994) and Zhao et al. (1997). dR stands for deoxyribose.

human cells, two amino acid sequence homologs of the Drosophila (6-4) photolyase are found in the human genome (approximately 40-50% identity) (Hsu et al., 1996; van der Spek et al., 1996; Todo et al., 1997b). What are the functions of these (6-4) photolyase sequence homologs in humans? One hypothesis in the literature is that these human proteins act as photoreceptors through the protein-bound chromophores (Hsu et al., 1996), one of which has been identified as flavin. If indeed this is true, this would be a spectacular example of "recycling" in biology in that Mother Nature always finds a way to give new meaning to a polypeptide.

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