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Review Article

The Roles of Several Residues of *Escherichia coli* DNA Photolyase in the Highly Efficient Photo-Repair of Cyclobutane Pyrimidine Dimers

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Escherichia coli DNA photolyase is an enzyme that repairs the major kind of UV-induced lesions, cyclobutane pyrimidine dimer (CPD) in DNA utilizing 350–450 nm light as energy source. The enzyme has very high photo-repair efficiency (the quantum yield of the reaction is ~0.85), which is significantly greater than many model compounds that mimic photolyase. This suggests that some residues of the protein play important roles in the photo-repair of CPD. In this paper, we have focused on several residues discussed their roles in catalysis by reviewing the existing literature and some hypotheses.

1. Introduction

The sun gives warmth and light to the living beings on the earth. However, the ultra-violet (UV) radiation in the sunlight stimulates lesions forming in DNA. The UV-induced lesions in DNA block the replication and transcription events in the living cells, cause growth delay, mutagenesis, or lethal effects to organisms [1]. In order to survive under the sunlight, the organisms have evolved several repair mechanisms to resist the harmfulness of UV. Direct reversal by DNA photolyases is one of the mechanisms. There are two types of DNA photolyases, CPD photolyases and (6-4) photolyases, which, respectively, reverse the two major UV-induced lesions in DNA, cyclobutane pyrimidine dimers (CPD), and (6-4) photoproducts, utilizing blue or near-UV light (350–450 nm) as energy source [1–4]. CPD photolyases can be further categorized into two subclasses, class I (microbial) and class II (animal and plant), based on their amino acid sequence similarity [3, 5, 6]. Flavin adenine dinucleotide (FAD) is catalytic cofactor of all photolyases [3]. And a second cofactor, usually a derivative of folate, deazaflavin or flavin, acts as a photoantenna to increase the repair efficiency of the enzymes under limiting light conditions [3, 7–9]. The repair reactions are proposed through a photon-induced electron transfer mechanism which is supported by many model compounds studies. However, the quantum yields (Φ = 0.7–0.98) in the repair of pyrimidine dimers by DNA photolyase is significantly higher [3, 10–13] than those model compounds (Φ = 0.016–0.4) [14–16]. These results indicate that some amino acid residues of photolyases play important roles in the repair reactions.

Escherichia coli DNA photolyase is a representative of them. By reviewing the existing literature and some hypotheses, we discussed the roles of some residues of *E. coli* photolyase in the highly efficient catalysis. This paper would provide the further insights into the catalytic mechanism of the enzyme.

2. *Escherichia coli* DNA Photolyase

Escherichia coli DNA photolyase is a class I CPD photolyase [17], containing 471 amino acids [18, 19] and two cofactors, FAD [20] and a folate derivative, 5,10-methenyltetrahydropterolypolyglutamate (MTHF) [7]. The enzyme was found in 1950s by Rupert et al. [21]. Its gene was first cloned by Sancar et al. [18, 19], which settled the problem that the little expression of the gene in the cells prevents the high yield of pure enzyme for researches.

During the following years, the enzyme has been extensively studied. The physiological form of the enzyme contains a fully reduced FAD ($FADH^-$) that is required for its activity both *in vivo* and *in vitro* [22]. It binds a CPD in DNA independent of light [17] and flips the dimer out of the double helix into the active site cavity to make a stable enzyme-substrate complex [23–26]. The light-dependent catalytic reaction was proposed through these steps: $FADH^-$ is excited directly by a photon or by the photoexcited MTHF cofactor and then transfers an electron to CPD to generate a charge-separated radical pair ($FADH^\bullet + CPD^{\bullet-}$); then the CPD radical anion cleaves, and the excess electron returns to $FADH^\bullet$ to restore the reduced form and close the catalytic photocycle [3, 11, 22, 27–29]. By the techniques such as time-resolved spectroscopy, laser flash photolysis [30–35], and transient electron paramagnetic resonance [36, 37], this photon-induced electron transfer mechanism has been substantiated. However, the roles of the amino acid residues in the steps of the high efficient enzymatic reaction, such as substrate docking and splitting, electron transfer, and intermediate stabilization, need further investigation.

3. Trp277: A Residue for CPD Docking and Splitting

E. coli DNA photolyase contains 15 tryptophan residues. Trp277 lies in a highly conserved region Trp277-Tyr281, which is considered to be important for DNA binding [38]. By the site-directed mutagenesis studies, it was found when Trp277 was replaced with arginine or glutamate, the binding affinity for CPD substrate was lower for 300- or 1000-fold, respectively, although the photochemical properties and the quantum yields for catalyses (under the irradiation wavelengths at 366 nm and 384 nm) of the mutants were indistinguishable from the wild-type enzyme [38]. Later on, it was discovered that Trp277 can also directly and efficiently repair CPD under 280 nm light [39]. These results revealed that Trp277 is crucial for substrate binding, and under certain conditions it also acts as a catalytic residue.

The crystal structure of *E. coli* photolyase (Protein Data Bank entry 1DNP) shows that a positively charged groove on the surface of the protein which might interact with the DNA backbone and a hydrophobic cavity locates at the center. The cavity has the right dimension to hold a *cis,syn* CPD, and Trp277 forms one side wall of it [40] (Figure 1(a)). It is proposed that photolyase binds DNA chain containing a CPD, flips it out into the cavity, and Trp277 stacks with the 5′ side of the CPD by π-π interaction [23–26, 38]. This is confirmed by the crystal structure of the complex of CPD-like lesion in DNA and photolyase from *Anacystis Nidulans* (*Synechococcus* sp.) (Protein Data Bank entry 1TEZ) [41].

There is another tryptophan, Trp384, in the cavity forming a wedge with Trp277 (Figure 1(a)) [40]. By the examination of the cocrystal structure of *Thermus thermophilus* photolyase with a thymine, it is proposed that CPD might be sandwiched by these two tryptophans [42], and its 3′ side might stack with Trp384 in a similar manner. However, from the structure 1TEZ, it is concluded that the 3′ side should stack with a methionine residue, Met345, but not Trp384. Met345 is to be discussed in the next section.

4. Met345: A Discriminating Residue of CPD Photolyase from Photolyase-Cryptochrome Super Family

A methionine residue in the active cavity of *Saccharomyces cerevisiae* photolyase, which corresponds to Met345 of *E. coli* photolyase, was predicted to interact with CPD [24]. By the structure of the complex of *Anacystis Nidulans* photolyase and CPD-like lesion, it was confirmed that Met345 should stack with the 3′ side of CPD [41] (Figure 1(a)). Methionine is a sulfur-containing amino acid. From the studies of the crystal structures of many proteins, Morgan and coworkers proposed that the sulfur atoms might interact with aromatic rings by the so-called sulfur-π interaction [43–51]. Although the mechanism of this interaction is still controversial, it does exist. For example, in the structure of the flavodoxin of *Clostridium beijerinckii* (Protein Data Bank entry 5ULL), a methionine residue is located near the xylene ring side of the flavin cofactor [52] the conformations are just like those of Met345 and the 3′ side of CPD (Figure 1(b)). The sulfur-π stack might also contribute to substrate-binding affinity. In addition, this interaction together with that between Trp277 and the 5′ side of CPD might have some effects on substrate splitting.

The electron transfer from excited $FADH^-$ to CPD is now considered to be through a direct pathway [33, 53, 54]. However, a theoretical calculation with the CPD-photolyase complex structure shows that the indirect electron transfer via protein mediators is as important as the direct electron transfer [55]. The electron-tunneling pathway analysis suggested that there were two typical electron-tunneling routes for the electron transfer of photolyase, one was an adenine route, and the other was through a methionine corresponding to Met345 of *E. coli* photolyase [55]. It is widely accepted that the electron transfers from $FADH^-$ to the 5′ side of CPD first, then to the 3′ side [3, 32]. And the pathway for excess electron transfer back to $FADH^\bullet$ remains unclear till now. Considering Met345 adjacent to the 3′ side of CPD, we speculate that it might be an electron back transfer pathway.

It is intriguing that Met345 is proposed to be a residue for the discrimination of CPD photolyase from the photolyase-cryptochrome super family [55]. The super family contains CPD photolyases, (6-4) photolyases, and cryptochromes, all of which are flavoproteins. (6-4) photolyases repair (6-4) photoproduct but not CPD [4]. Cryptochromes play roles in photomorphogenesis in plants and entrain the circadian biological clocks in animal [2, 3, 56]. Recently, it is found that some cryptochromes in insects and birds might function as light-activated magnetoreceptors [57–61]. Although these proteins are functionally diverse, they have relatively high degree of homology. Met345 is conserved in all CPD photolyase whereas in (6-4) photolyases, it is replaced by a histidine which is also important for catalysis [62]. In cryptochromes, it is replaced by histidine, valine, glutamine, and so forth. It might be one of the residues responsible for

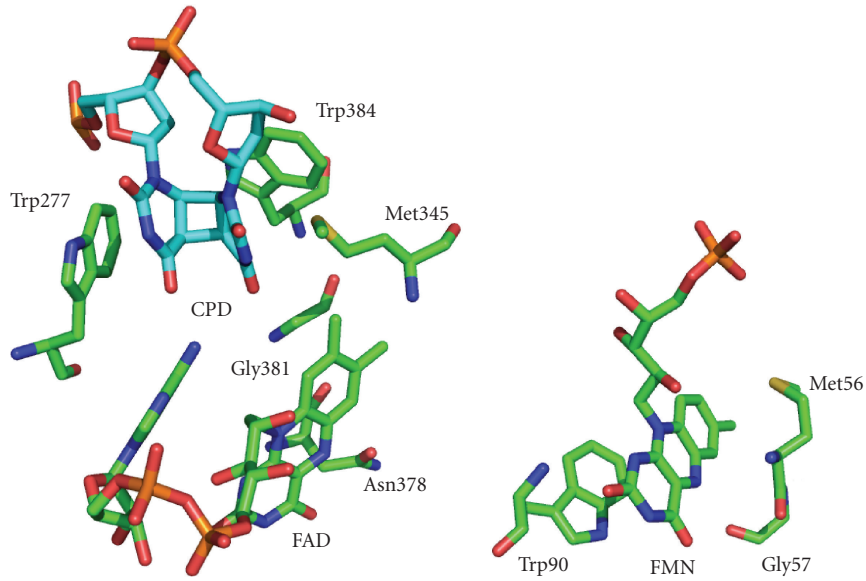

Figure 1: Conformations of (a) active sites of photolyase and (b) FMN binding sites of *Clostridium beijerinckii* flavodoxin. The CPD structure was extracted from the PDB entry 1SNH and coupled with *E. coli* DNA photolyase structure 1DNP according to the CPD like lesion-photolyase complex structure 1TEZ. The FMN binding sites of flavodoxin were extracted form the PDB entry 5ULL. This figure was prepared with PyMOL (http://www.pymol.org/).

the function diversity of the proteins in this super family [55].

5. Asn378: A Stabilizer of the Neutral FAD Radical

Although *E. coli* photolyase contain reduced FAD *in vivo*, it is usually purified with FAD in the blue neutral radical form ($FADH^{\bullet}$). It is known that *E. coli* photolyase is one of the unusual flavoproteins in which the radical is extremely stable [63]. The purified enzyme can hold its radical flavin cofactor unoxidized in aerobic conditions for several days whereas it hardly exists free in aqueous solutions because the dismutation of the radical is favored. These results indicate that the protein environment gives a strong stabilization to the radical. *Clostridium beijerinckii* flavodoxin is another example that has the ability to hold stable radical flavin cofactor. It was proposed that a hydrogen bond between the flavin N(5)H group and the backbone carbonyl oxygen of Gly57 in the flavodoxin is important for the modulation of the redox potentials of the cofactor and the stabilization of the radical form (Figure 1(b)) [64, 65]. Interestingly, there is a similar hydrogen bond between the flavin N(5)H group and the side carbonyl of the Asn378 residue (Figure 1(a)). We had replaced the asparagine residue with serine and found that the mutant has no stable radical state [66]. Moreover, the catalytic activity of the mutant was lost [66]. These experiments show that Asn378 is crucial both for the stabilizing the neutral flavin radical cofactor and for catalysis. It is convincible because the catalytic reaction of CPD splitting is through a radical mechanism: $FADH^{-}$ gives an electron to CPD and becomes $FADH^{\bullet}$. If the transient radical intermediate is well stabilized, it will give enough time for cleavage of the cyclobutane ring to achieve high repair efficiency [33, 67]. When the stabilizing effect is disrupted, the unwanted back electron transfer might be accelerated, leading to low repair efficiency.

Asn378 is a highly conserved residue in photolyases. In most class I CPD photolyases and (6-4) photolyases, the residues are unchanged or replaced with asparate [6, 68]. The residue is also conserved in mammalian cryptochromes [69]. In a plant cryptochrome *Arabidopsis thaliana* CRY1, it is replaced with asparate that is proposed to be responsible for the down shift of the flavin redox potentials which make the difference between the cryptochrome and photolyases [70]. Meanwhile, some insect cryptochromes have replaced the residue with cysteine, which may be responsible for a red anionic radical ($FAD^{\bullet-}$) state but not the blue neutral radical state in photolyases [69, 71]. However, in many class II CPD photolyases, the asparagine residues are not conserved [6, 72]. There is evidence that class II CPD photolyases have the similar photochemical properties and the FAD binding environments as compare to class I CPD and (6-4) photolyases [72]. Thus, a stabilizer near the flavin N(5)H group is also required in a class II enzyme. By examining a model structure of a class II enzyme (*Oryza sativa* CPD photolyase) calculated by comparative modeling (http://modbase.compbio.ucsf.edu/modbase-cgi/index.cgi) [72, 73], we find another asparagine residue, Asn421, might be an alternative candidate for the stabilization function, which is also highly conserved in class II CPD photolyases.

From the model structure of *Oryza sativa* CPD photolyase, it is of interest to find that the residue corresponding to Trp277 in *E. coli* photolyase is not conserved, which is considered to be crucial for substrate binding (*vide ante*). It indicates that the class II CPD photolyases might use a different substrate binding mechanism as compared to class I group. However, it seems that the residues corresponding to Met345 and Trp384 in *E. coli* photolyase are conserved in the substrate binding cavity, emphasizing their important roles for substrate binding and/or catalysis. Therefore, to fully uncover details of substrate binding and FAD usage, a real crystal structure of a class II photolyase is highly awaited [72].

A mutagenesis study on *Anacystis nidulans* photolyase shows that two residues (Trp384 and Gly381 of *E. coli* photolyase) are crucial for the kinetics stability of the neutral flavin radical in the enzyme (Figure 1(a)) [74]. In *Synechocystis* sp. PCC6803 CRY-DASH, these residues are replaced with tyrosine and asparagine, respectively [74]. The difference might also be a reason for the diverse functions of photolyases and cryptochromes.

6. Perspectives

Although the first photolyase has been discovered more than 50 years [21], photolyases still occupies a unique position in biochemistry [1, 2]. Photolyases bind the substrate in a light-independent manner, but the catalysis is absolutely dependent on light that makes the possible to analyze the binding and catalysis steps of the enzymes independently [2, 3]. Furthermore, the level of substrate in the cell can be controlled easily by simply changing the UV dose, and the repairing of the bound substrate is ultrafast by a single light flash. All characteristics make the photolyases to be useful tools for biochemical research, especially the *in vivo* enzymology [2]. Cryptochromes are homologues of photolyases, which are widely concerned now for their functions in the circadian clocks of animal, the photomorphogenesis of plants and the migration of birds and insects by acting as light-activated magnetoreceptors [2, 3, 56–61]. The action mechanisms of cryptochromes are still unclear at present. However, as photolyases, flavin radical is also proposed to be crucial for their functions [57–61, 69–71, 74–76]. Thus, further exploring the photolyase system might give new insights into the researches of both photolyases and cryptochromes.

7. Summary

In this paper, we have discussed some of important residues of *E. coli* DNA photolyase. Evidence suggests that they play significant roles in substrate docking and splitting, electron transfer, and intermediate stabilization. Of course, due to the limits of our knowledge, there must be a lot of amino acid residues that might be much more important in the catalysis of enzyme, which are not discussed here. Together with all these amino acid residues and the cofactors of the enzyme, they built a very high efficient system for the photo-repair of cyclobutane pyrimidine dimers in DNA. Further research on this system will not only contribute to understanding its efficient catalytic mechanism, but also give new insights to the other biochemical research in the future.

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