Published in final edited form as:

Science. 2016 October 14; 354(6309): 209–213. doi:10.1126/science.aah6071.

Bifurcating electron-transfer pathways in DNA photolyases determine the repair quantum yield

Meng Zhang¹, Lijuan Wang¹, Shi Shu¹, Aziz Sancar², Dongping Zhong^{1,*}

¹Department of Physics, Department of Chemistry and Biochemistry, and Programs of Biophysics, Chemical Physics, and Biochemistry, The Ohio State University, Columbus, OH 43210, USA.

²Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.

Abstract

Photolyase is a blue-light-activated enzyme that repairs ultraviolet-induced DNA damage that occurs in the form of cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone (6–4) photoproducts. Previous studies on microbial photolyases have revealed an electron-tunneling pathway that is critical for the repair mechanism. In this study, we used femtosecond spectroscopy to deconvolute seven electron-transfer reactions in 10 elementary steps in all classes of CPD photolyases. We report a unified electron-transfer pathway through a conserved structural configuration that bifurcates to favor direct tunneling in prokaryotes and a two-step hopping mechanism in eukaryotes. Both bifurcation routes are operative, but their relative contributions, dictated by the reduction potentials of the flavin cofactor and the substrate, determine the overall quantum yield of repair.

Photolyases, which belong to the photolyase (PL)–cryptochrome (CRY) superfamily, use a fully reduced flavin (FADH[−]) cofactor to repair sunlight-induced DNA lesions, including cyclobutane pyrimidine dimers (CPDs) and pyrimidine-pyrimidone (6–4) photo-products (1–5). On the basis of sequence analyses, CPD photolyases are highly diversified and can be subdivided into three classes (I to III) (6–8), mechanism recently obtained from the well-characterized class I photolyase from *Escherichia coli* (EcPL) (9, 10) may not be applicable to other subfamilies, especially the distant class II PLs. We used ultrafast spectroscopy to systematically investigate class I AnPL (from *Anacystis nidulans*), class II DmPL (from *Drosophila melanogaster*) and AtPL (from *Arabidopsis thaliana*), and class III CcPL (from *Caulobacter crescentus*) and ssDNA AtPL (AtCRY3) to search for conserved features of the overall photolyase repair mechanism.

*Corresponding author.: zhong.28@osu.edu.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/354/6309/209/suppl/DC1

Figs. S1 to S4

Tables S1 to S4

References (31–36)

Class II PLs have Gly and Tyr or Phe residues at two positions near the dimer substrate in the active site that are occupied, respectively, by Asn and Arg residues in the other PLs, as well as one Asn residue near the N5 position of the flavin cofactor that is contributed by a different helix (Fig. 1, B and D). Otherwise, the overall active-site configuration is conserved across all PLs, especially the folded structure of the flavin cofactor with the adenine moiety in the middle between the tricyclic ring and the dimer substrate (Fig. 1D) (11–15). The different residues in the flavin and substrate binding pockets alter the reduction potentials of the dimer and the cofactor. In particular, the substitution of Gly in place of Asn creates a space filled in by several water molecules (Fig. 1D) (11, 12). Such reduction-potential changes could lead to distinct reaction dynamics that would account for the wide distribution of total repair quantum yields (QYs) observed across the different classes of PLs (Fig. 1C). Here we present the key results for the two distant class I and class II PLs and then summarize our findings, as well as similar observations for other PLs that are also listed in the supplementary materials.

To analyze the CPD repair dynamics, we first measured the absorption transients of the PLs without substrate probed at 800 nm (Fig. 2A) and 270 nm (Fig. 2B) after excitation at 400 nm (Fig. 2D). Previous studies (16) revealed dynamics attributed to an intramolecular electron transfer (ET) between the tricyclic ring (LfH^{*-}) and the adenine moiety (Ade) in the conserved flavin folded structure [forward electron transfer 1 (FET1) (Fig. 2D)] and two subsequent back electron transfers (BETs) to the initial excited state (BET1a) and the original ground state (BET1b). The 800-nm transients in Fig. 2A reflect the pure excited LfH^{*-} dynamics, whereas the 270-nm transients in Fig. 2B mainly capture the intermediate $\text{LfH}^{\bullet}$ semiquinone dynamics (16), which vary across the three PLs. Together with other wavelength detection (fig. S2), we systematically analyzed these stretched dynamics that arise from the coupling with the active-site relaxation (9, 17). We then derived the initial charge separation (FET1) for AnPL, DmPL, and AtPL in average times of 1340, 1590, and 564 ps, respectively; the reverse charge recombination (BET1a) in 1230, 1029, and 1505 ps, respectively; and the back electron transfer (BET1b) to the ground state in 26, 715, and 17 ps, respectively, with a nearly constant deactivation lifetime of 6 ns (see supplementary materials and table S1). Both AnPL and AtPL promptly return to the ground state via ultrafast BET1b, whereas DmPL exhibits an apparent long decay (Fig. 2A) because the forward electron transfer for DmPL (FET1 = 1590 ps) is similar to that of AnPL (FET1 = 1340 ps). Thus, the slow DmPL transient in Fig. 2A is caused by substantial reverse charge recombination BET1a to regenerate LfH^{*-} because of much slower BET1b (715 ps). These dynamics are critical to determining the repair pathways and, therefore, the QYs of different classes of photolyases.

We next measured 800-nm absorption transients of the three enzyme-substrate complexes after 400-nm excitation (Fig. 2C; the dashed lines are reproduced from Fig. 2A for comparison). Notably, the dynamics in the three PL-substrate complexes of the three photolyases are considerably different. AnPL, similar to the class I EcPL (18–20), displays a much faster decay in the presence of the substrate, indicating that the electron flows to the substrate efficiently: FET2 = 209 ps (Fig. 2D), which is substantially shorter than the bifurcated FET1 pathway (1340 ps). Thus, the repair mechanism is similar to that observed for the same class EcPL. The electron primarily tunnels directly to the substrate with a

favorable, negative free energy, mediated by the intervening adenine in the fold structural motif (18–21). For DmPL, the overall dynamics in the complex becomes slightly faster if we consider only the forward FET1 process (1590 ps), resulting in an ET time of 5315 ps (FET2), which is much longer than the 1590 ps of FET1 for the electron transfer to the adenine moiety. Thus, the electron path bifurcates (Fig. 2D): Some electrons (19%) tunnel to the substrate through the adenine, whereas others (64%) hop to the adenine to form the anion Ade^- intermediate (the remaining 17% goes to the ground state via deactivation; see table S2). Surprisingly, for AtPL the excited flavin dynamics are nearly the same as observed without the substrate with a FET1 time of 564 ps. Best-fitting of the transient indicates a tunneling ET directly to the substrate in 6500 ps (FET2), which indicates that the electron dominantly hops to the adenine, a situation completely opposite of that in class I AnPL and EcPL. Thus, the structural and chemical properties in class II PLs favor electron hopping to, rather than electron tunneling through, the intervening adenine. From x-ray structures (11–15), the edge-to-edge distance between the adenine and substrate CPD (Fig. 2D) is about 3 Å and, therefore, the further electron hopping (FET3 in Fig. 2D) from the anion adenine to CPD is ultrafast (see below), given the negative free energy of favorable reduction potentials (table S3).

After discovering the initial electron-transfer pathways, we probed the repair dynamics comprehensively from the visible to the deep ultraviolet (UV) region to completely resolve the reaction intermediates as we did in studies of EcPL (19) (Fig. 3, A to H). These dynamics show distinct profiles. With systematic analyses, we can dissect each transient into distinct components, from initial reactants to various intermediates and final products. The repair follows the reaction scheme in Fig. 2D: After the electron from $\text{LfH}^{\bullet*}$ finally arrives in the CPD, the first C–C bond breakage is ultrafast in less than 10 ps (19, 22), and the second C–C bond cleavage (SP) occurs in tens of picoseconds (19, 23). Finally, the electron returns to the flavin semiquinone ($\text{LfH}^{\bullet}$) to close the entire photocycle. Specifically, in the visible region at 510 nm (Fig. 3A), we detected only the cofactor flavin signal with two components of the excited ($\text{LfH}^{\bullet*}$) and intermediate ($\text{LfH}^{\bullet}$) states (Fig. 3B). Note that the $\text{LfH}^{\bullet}$ signal is a summation of contributions of three components (see equation S12 and fig. S3 in the supplementary materials). In the UV region (Fig. 3, C to H), the transients contain all of the flavin signals, including those of the excited state of $\text{LfH}^{\bullet*}$, the immediate states of $\text{LfH}^{\bullet}$ and Ade^- , and the final states of LfH^- and Ade , as well as the overall substrate signals of the intermediate T-T^- after the first C–C bond breakage, T^- after the second C–C cleavage, and the final product of repaired base T. The various dissections are shown in Fig. 3, D, F, and H, and fig. S4. Thus, we obtained the ultrafast electron hopping of FET3 in 6, 11, and 15 ps and the electron return after repair in 437, 2890, and 819 ps for AnPL, DmPL, and AtPL, respectively. Knowing the total QYs (Fig. 1C), we can also derive the second C–C cleavage in 87, 48, and 36 ps and the futile back electron transfer BET2 in 1138, 149, and 527 ps, respectively, for three PLs (table S1).

To recapitulate, we have identified 10 elementary steps in the repair reaction by DNA photolyase, including 7 ET steps, and measured their time scales in real time (table S1). Consequently, we can calculate the QY of each step that contributes to the total QY (table S2). In Fig. 4, A and B, we show the two resolved photocycles for class I AnPL and class II AtPL, respectively, with the corresponding reaction times of each step. For class I PL (Fig.

4A), the two systems we studied, AnPL and EcPL, show a dominant tunneling pathway with the highest QYs (table S2). For class II PL (Fig. 4B), the two systems studied here, DmPL and AtPL, adopt mainly a two-step hopping route, also with good repair efficiency. For other PLs [class III CcPL and ssDNA-specific AtPL (AtCRY3)], both tunneling and hopping channels are operative (table S1). These detailed dynamics and time scales for seven ET reactions involved in repair can be used to derive microscopic pictures of various reorganization energies; their relevant reduction potentials; and, thus, reaction driving forces (table S3) (21, 24, 25). We did not observe clear evidence for the possible flickering resonance for the initial electron bifurcation, as proposed recently in a theoretical study (26).

Figure 4C shows the repair QYs along the evolutionary path from the microbial class I to the eukaryotic class II PLs, with initial electron bifurcation into the tunneling route FET2 and the hopping path FET1 and their resulting QYs (QY2 and QY1). Clearly, the tunneling route in class I leads to a higher repair QY. With the decrease in the rates of tunneling, the hopping channel comes to dominate in class II PLs. Consequently, class II PLs can never reach the class I repair QY because the electron path at Ade^- also bifurcates into the repair channel to the CPD and the futile path back to the original ground state, both of which share similar hopping rates. The conserved active-site configuration and the folded flavin structure that occur as a result of evolution in the entire photolyasecryptochrome superfamily (11–15, 27–30) are essential to ensure a unified electron-transfer mechanism through electron path bifurcation into two operative routes for all CPD photolyases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We dedicate this paper to the memory of the “father of femtochemistry,” Ahmed H. Zewail, who passed away on 2 August 2016. D.Z. was trained as a student and later as a postdoctoral fellow in Dr. Zewail’s lab. We thank Y.-T. Kao, Z. Liu, X. Guo, C. Tan, Y. Qin, and N. Ozturk for help in the initial stages of this work. We also thank P. Houston for careful reading of the manuscript. This work was supported, in part, by NIH grants GM074813 and GM118332 to D.Z. and GM031082 to A.S. Additional data supporting the conclusions of this study are included in the supplementary materials.

REFERENCES AND NOTES

1. Sancar A, Chem. Rev 103, 2203–2238 (2003). [PubMed: 12797829]
2. Lin C, Todo T, Genome Biol 6, 220 (2005). [PubMed: 15892880]
3. Kim ST, Sancar A, Essenmacher C, Babcock GT, Proc. Natl. Acad. Sci. U.S.A 90, 8023–8027 (1993). [PubMed: 8396257]
4. Brash DE, Trends Genet. 13, 410–414 (1997). [PubMed: 9351343]
5. Taylor JS, Acc. Chem. Res 27, 76–82 (1994).
6. Kanai S, Kikuno R, Toh H, Ryo H, Todo T, J. Mol. Evol 45, 535–548 (1997). [PubMed: 9342401]
7. Sancar A et al., Cold Spring Harb. Symp. Quant. Biol 65, 157–172 (2000). [PubMed: 12760030]
8. Lucas-Lledó JI, Lynch M, Mol. Biol. Evol 26, 1143–1153 (2009). [PubMed: 19228922]
9. Zhong D, Annu. Rev. Phys. Chem 66, 691–715 (2015). [PubMed: 25830375]
10. Liu Z, Wang L, Zhong D, Phys. Chem. Chem. Phys 17, 11933–11949 (2015). [PubMed: 25870862]
11. Kiontke S et al., EMBO J. 30, 4437–4449 (2011). [PubMed: 21892138]

12. Hitomi K et al., *J. Biol. Chem* 287, 12060–12069 (2012). [PubMed: 22170053]
13. Park HW, Kim ST, Sancar A, Deisenhofer J, *Science* 268, 1866–1872 (1995). [PubMed: 7604260]
14. Mees A et al., *Science* 306, 1789–1793 (2004). [PubMed: 15576622]
15. Huang Y et al., *Proc. Natl. Acad. Sci. U.S.A* 103, 17701–17706 (2006). [PubMed: 17101984]
16. Liu Z et al., *Proc. Natl. Acad. Sci. U.S.A* 110, 12972–12977 (2013). [PubMed: 23882072]
17. Chang C-W et al., *Proc. Natl. Acad. Sci. U.S.A* 107, 2914–2919 (2010). [PubMed: 20133751]
18. Kao YT, Saxena C, Wang L, Sancar A, Zhong D, *Proc. Natl. Acad. Sci. U.S.A* 102, 16128–16132 (2005). [PubMed: 16169906]
19. Liu Z et al., *Proc. Natl. Acad. Sci. U.S.A* 108, 14831–14836 (2011). [PubMed: 21804035]
20. Tan C et al., *Nat. Commun* 6, 7302 (2015). [PubMed: 26065359]
21. Liu Z et al., *J. Am. Chem. Soc* 134, 8104–8114 (2012). [PubMed: 22533849]
22. Hassanali AA, Zhong D, Singer SJ, *J. Phys. Chem. B* 115, 3848–3859 (2011). [PubMed: 21417374]
23. Thiagarajan V, Byrdin M, Eker AP, Müller P, Brettel K, *Proc. Natl. Acad. Sci. U.S.A* 108, 9402–9407 (2011). [PubMed: 21606324]
24. Wang H et al., *Science* 316, 747–750 (2007). [PubMed: 17478721]
25. LeBard DN, Kapko V, Matyushov DV, *J. Phys. Chem. B* 112, 10322–10342 (2008). [PubMed: 18636767]
26. Zhang Y, Liu C, Balaeff A, Skourtis SS, Beratan DN, *Proc. Natl. Acad. Sci. U.S.A* 111, 10049–10054 (2014). [PubMed: 24965367]
27. Maul MJ et al., *Angew. Chem. Int. Ed* 47, 10076–10080 (2008).
28. Xing W et al., *Nature* 496, 64–68 (2013). [PubMed: 23503662]
29. Zoltowski BD et al., *Nature* 480, 396–399 (2011). [PubMed: 22080955]
30. Brautigam CA et al., *Proc. Natl. Acad. Sci. U.S.A* 101, 12142–12147 (2004). [PubMed: 15299148]

Fig. 1. Classes of photolyases, sequence alignment, steady-state repair QYs, and local structures. (A) Unrooted phylogenetic tree of the PL-CRY protein family and representative members. The class II PL is distant from the other subfamilies. (B) Sequence alignment of eight photolyases of different classes. Ec, *Escherichia coli*; An, *Anacystis nidulans*; Cc, *Caulobacter crescentus*; At, *Arabidopsis thaliana*; Mm, *Methanosarcina mazei*; Dm, *Drosophila melanogaster*; Os, *Oryza sativa*. Three conserved active-site residues (R, E, and M or Q) near the substrate are highlighted in yellow. Three other critical active-site residues that vary between the class I PLs and the other subfamilies are high lighted in blue. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (C) Enzyme activities and steady-state repair QYs of EcPL (as a control), AnPL, DmPL, AtPL, CcPL, and AtCRY3 (ssDNA AtPL). Every data point was averaged over five measurements, with error bars showing 1 SD of uncertainty. (D) Local active-site structures of class I AnPL (silver) and class II MmPL (light blue), highlighting the six critical residues and the catalytic flavin cofactor. In class II PL, a glycine residue (G375 in MmPL) replaces asparagine (N349 in AnPL).

Fig. 2. Photoinduced intramolecular electron transfer in photolyases and initial electron-transfer bifurcation in repair complexes.

(A) Absorption transients (A) of AnPL, DmPL, and AtPL enzymes probed at 800 nm for the excited-state flavin ($\text{Lfh}^{\bullet-}$). λ_{pr} , probe wavelength. (B) Absorption transients of AnPL, DmPL, and AtPL enzymes probed at 270 nm, mainly for the intermediate-state flavin radical ($\text{Lfh}^{\bullet}$). (C) Absorption transients of the three enzymes (dashed line) and enzyme-substrate complexes (solid lines) probed at 800 nm. In the presence of substrate, the excited-state dynamics of AnPL becomes drastically faster due to direct electron tunneling to the CPD substrate, whereas in class II photolyases the change of the excited-state dynamics is much smaller and is almost negligible in AtPL. (D) Repair scheme with seven electron-transfer reactions and two dimer-splitting processes among 10 elementary steps. A cyclic electron transfer between the tricyclic ring (lumiflavin, Lf) and adenine (Ade) is intrinsic in all photolyases (gray arrows in the molecular structure). Because the free energy of charge separation is close to zero, the excited and charge-separated states also interconvert. When the substrate is present, the electron path can bifurcate, either by direct tunneling to CPD through the intervening adenine via a superexchange mechanism or by a two-step hopping mechanism also bridged by the adenine (blue arrows). 1, $\text{Lfh}^{\bullet-}$ -Ade+T $\leftrightarrow$ T(CPD); 2, $\text{Lfh}^{\bullet-}$ -Ade+T $\leftrightarrow$ T; 3, $\text{Lfh}^{\bullet}$ -Ade $^{\bullet-}$ +T $\leftrightarrow$ T; 4, $\text{Lfh}^{\bullet}$ -Ade+T $\leftrightarrow$ T $^{\bullet-}$; 5, $\text{Lfh}^{\bullet}$ -Ade+T+T $^{\bullet-}$; 6, $\text{Lfh}^{\bullet}$ -Ade+T+T. h , Planck's constant; ν , frequency; SP, C-C bond splitting; ER, electron return.

Fig. 3. Absorption transients of enzyme-substrate complexes probed widely from the visible to the UV region to detect various intermediates and final products involved in repair.

(A and B) Absorption transients of AnPL, DmPL, and AtPL complexes probed at 510 nm (A) and the deconvolution of LfH^{*-} (dark green dashed line) and LfH^* (dark red dashed line) contributions from DmPL (B). (C to H) Absorption transients of the three complexes probed in the UV region mainly for detection of the thymine intermediates and final products. These dynamics are systematically fitted by the total flavin-related species (LfH^{*-} , LfH^* , LfH^- , Ade, and Ade^- , dashed orange line), the thymine dimer intermediate T-T^- (dashed magenta line), the thymine anion T^- (dashed light blue line), and the thymine product T (dashed dark yellow line), as shown in three deconvolution plots [(D), (F), and (H)].

Fig. 4. Complete photocycles of CPD repair by class I and class II PLs and QY evolution with bifurcated ET channels in different photolyases.

Repair cycles of (A) class I AnPL and (B) class II AtPL with seven ET reactions among 10 resolved elementary steps. In AnPL, FET2 is much faster than FET1, so the direct electron-tunneling channel is dominant. In AtPL, FET1 is much faster than FET2, and hence the two-step hopping pathway becomes dominant. (C) Changes in bifurcated ET rates for FET2 and FET1 and the resulting final QYs of the two respective paths, QY2 and QY1, ordered from microbial to eukaryotic PLs.