Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 8023–8027, September 1993
Biophysics

Time-resolved EPR studies with DNA photolyase: Excited-state $FADH^0$ abstracts an electron from Trp-306 to generate $FADH^-$, the catalytically active form of the cofactor

(ultraviolet/pyrimidine dimer/heavy isotopes)

SANG-TAE KIM*, AZIZ SANCAR*, CRAIG ESSENMACHER†, AND GERALD T. BABCOCK†

*Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, NC 27599; and †Department of Chemistry, Michigan State University, East Lansing, MI 48824

Communicated by JoAnne Stubbe, May 18, 1993

ABSTRACT **Photolyase repairs UV-induced cyclobutane-pyrimidine dimers in DNA by photoinduced electron transfer. The enzyme isolated from *Escherichia coli* contains 5,10-methenyltetrahydrofolate, which functions as the light-harvesting chromophore, and fully reduced flavin adenine dinucleotide (FAD), which functions as the redox catalyst. During enzyme preparation, the flavin is oxidized to $FADH^0$, which is catalytically inert. Illumination of the enzyme with 300- to 600-nm light converts the flavin to the fully reduced form in a reaction that involves photooxidation of an amino acid in the apoenzyme. The results of earlier optical studies had indicated that the redox-active amino acid in this photoactivation process was tryptophan. We have now used time-resolved electron paramagnetic resonance (EPR) spectroscopy to investigate the photoactivation reaction. Excitation of the flavin-radical-containing inactive enzyme produces a spin-polarized radical that we identify by ^2H and ^{15}N labeling as originating from a tryptophan residue, confirming the inferences from the optical work. These results and Trp → Phe replacement by site-directed mutagenesis reveal that flavin radical photoreduction is achieved by electron abstraction from Trp-306 by the excited-state $FADH^0$. Analysis of the hyperfine couplings and spin density distribution deduced from the isotopic-labeling results shows that the product of the light-driven redox chemistry is the Trp-306 cation radical. The results strongly suggest that the active form of photolyase contains $FADH^-$ and not $FADH_2$.**

DNA photolyase binds to *cis, syn*-pyrimidine dimer in UV-damaged DNA and splits the cyclobutane ring in the dimer by converting light energy into chemical energy (1). Photolyases contain two noncovalently bound chromophores. One is flavin adenine dinucleotide (FAD; refs. 2 and 3) and the other, commonly known as "the second chromophore," is either 5,10-methenyltetrahydrofolate (CH-THF; ref. 4) or 7,8-didemethyl-8-hydroxy-5-deazaflavin (5, 6). The second chromophore is a light-harvesting molecule with high extinction coefficient in the near UV–visible range (300–500 nm). The second chromophore absorbs a photon and transfers its excitation energy to the flavin (7, 8), which then splits the pyrimidine dimer by an electron-transfer process (9, 10).

In photolyase, the flavin cofactor can exist in an oxidized, a one-electron-reduced (neutral radical), or a two-electron-reduced state. The characteristic blue color of purified *Escherichia coli* photolyase arises from the one-electron-reduced state of flavin ($FADH^0$). This form of the enzyme binds substrate with normal affinity but is catalytically inactive (11, 12). However, excitation of the flavin radical either directly or through energy transfer from CH-THF results in one-electron reduction of the radical to the fully reduced active form. Previous work has shown that absorption of a photon by the ground state of $FADH^0$ yields the excited doublet state of the radical, which then undergoes intersystem crossing to the quartet state within 100 ps (13). The long-lived $FADH^0$ quartet is then reduced by a neighboring amino acid residue (14). In the absence of exogenous donors, a dark reversal of the flavin photoreduction occurs over 200 ms. Laser flash-photolysis studies suggested that the intrinsic reductant was a tryptophan residue that reduced the flavin radical by H-atom transfer (14). This conclusion was supported by a site-specific mutagenesis study in which each of the 15 tryptophan residues of photolyase was replaced by phenylalanine, individually. The Trp-306 → Phe (W306F) mutation abolished photoreduction of $FADH^0$ without affecting the substrate binding of the enzyme (15), thus providing evidence that Trp-306 is the internal photoreductant of $FADH^0$. Time-resolved EPR studies on the photoreduction of $FADH^0$ have confirmed that tryptophan is the internal reductant (16). Our initial magnetic resonance study (16) showed that flash photolysis of the $FADH^0$-containing inactive enzyme produced a radical intermediate and that the hyperfine structure of the radical was partially collapsed when the α protons of the indole ring were replaced by ^2H. This result provides unambiguous confirmation of the optical studies and shows that tryptophan is the internal reductant in the photoreduction process. Thus, the data obtained by three independent methods are consistent in establishing tryptophan involvement in photoreduction. Remaining to be resolved, however, are key aspects of the photoreduction process. Whether the photochemistry proceeds by electron transfer or H-atom transfer is unknown, although H-atom transfer to produce a neutral tryptophan radical has been postulated (14); moreover, the protonation state of the flavin chromophore following photoreduction, both transiently and in the final, active enzyme, is controversial.

In this study, we have used isotopic labeling, flash photolysis, and time-resolved EPR in order to generate and identify the radical intermediate(s) involved in the reduction of $FADH^0$. From the time-resolved EPR analyses of wild-type and mutant photolyase containing isotopically labeled tryptophan we provide additional evidence that Trp-306 is the endogenous reductant of the apoenzyme involved in the flavin radical reduction. Most importantly, our results suggest that photoreduction of flavin radical is achieved by electron transfer, and not H transfer, which in turn implies that the catalytically active form of the reduced flavin in photolyase is the anionic form ($FADH^-$) rather than the dihydro form ($FADH_2$).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CH-THF, 5,10-methenyltetrahydrofolate; Enz-$FADH^0$, photolyase containing flavin radical and depleted of CH-THF; MO, molecular orbital.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The bacterial strains used were derivatives of *E. coli* K-12. PLK983 (*trp*$^-$) was obtained from the *E. coli* Genetic Stock Center, Yale University. MS09, a photolyase-overproducing strain, has been described (17). pMS969 is a photolyase-overproducing plasmid (17).

Heavy Isotope Labels. L-Tryptophan (*indole*-^{15}N, 99%) was obtained from Cambridge Isotope Laboratories (Cambridge, MA). The ring-deuterated tryptophans (*indole*-d_5; *indole*-2,5-d_2) were prepared from tryptophan by refluxing in 0.2 M $^2HCl/^2H_2O$ for 3 or 20 days under an argon-saturated atmosphere (18). The $^2HCl/^2H_2O$ solution was changed several times over the course of the experiment. The progress of the deuterium incorporation was monitored by 400-MHz ^1H NMR. For tryptophan-d_2, deuterium was incorporated up to 98% and 96% into the C-2 and C-5 positions, respectively. For tryptophan-d_5, deuterium incorporation was up to 99% in each position of the indole ring. Assignment of the deuterated positions was made on the basis of ^1H and ^{13}C NMR, and the deuterium contents were estimated by integration of the signals in the aromatic region in the 400-MHz ^1H NMR.

Growth Media and Conditions. MS09 was grown in Luria broth and PLK983/pMS969 was grown in K medium containing isotopically labeled tryptophan (2.5 μg/ml). Cells were grown an OD_{600} of 0.7, at which time isopropyl β-D-thiogalactopyranoside was added (1 mM) to induce the *phr* gene. After 12 hr of induction the cells were harvested and stored at −80°C until use.

Purification of Photolyase. Photolyase was purified by successive chromatography on blue Sepharose, Bio-Gel P100, and hydroxyapatite resins to >98% homogeneity (19). Enzyme containing only flavin and no CH-THF (Enz-FADH) was prepared by either selective photodecomposition (20) or $NaBH_4$ reduction (21) of CH-THF. After removal of CH-THF, the enzyme was dialyzed against 50 mM potassium phosphate, pH 7.0/50 mM NaCl/50% (vol/vol) glycerol and stored at −80°C until use.

EPR Measurements. EPR spectra were taken at 298 K in a quartz flat cell (Wilmad, Buena, NJ) with a Bruker ER-200D spectrometer operating at a microwave power of 6.3 mW, microwave frequency of 9.22226 GHz, and a modulation amplitude of 2.8 G (1 G = 10^4 T). Magnetic field strength and microwave frequency were monitored continuously by using a Bruker ER-035M gaussmeter and a Hewlett–Packard 5255A frequency converter/5245C counter, respectively. Transient EPR spectra were obtained by using gated integration techniques (16, 22) with a delay of 4 μs and an aperture of 48 μs. ac-coupling (ν > 10 Hz) was used to discriminate against any stable $FADH^0$ signals present. The time-resolved spectra were accumulated and averaged as indicated in the figure captions to improve the signal/noise ratio. The concentration of the enzyme was typically 0.1 mM. The photolysis reaction buffer contained 50 mM potassium phosphate (pH 7.0), 50 mM NaCl, 5 mM $K_3Fe(CN)_6$, 1 mM $K_4Fe(CN)_6$, and 10% glycerol. The samples were excited with 17-μs pulses from a Xenon flashlamp that was critically damped and operated at a repetition rate of 1 Hz. The electrical energy supplied to the lamp was 50 J.

RESULTS AND DISCUSSION

The flavin cofactor of most photolyases is oxidized to the semiquinone form during purification (3, 11). This form is inactive (3, 23) but is converted by photoreduction to the fully reduced form during illumination with photoreactivating light (11, 20, 23). Since transient photoreduction occurs in the absence of the second chromophore and of externally added reducing agents, the immediate electron donor must be an amino acid in the apoenzyme (20). Nanosecond flash photolysis studies by Heelis *et al.* (14, 20) revealed a transient with a broad absorption peak in the 500- to 600-nm region which was ascribed to a tryptophan neutral radical (14). This assignment was supported by a site-specific mutagenesis study that showed that the replacement of Trp-306—but not of any of the other 14 tryptophan residues—by phenylalanine made *E. coli* photolyase nonphotoreducible (15). However, the difference spectrum obtained by flash photolysis did not have enough resolution to discriminate unambiguously between tryptophan neutral and cation radicals, and the site-specific mutagenesis provided only negative evidence for Trp-306 being the intrinsic reductant.

The observation of catalytically relevant redox activity in amino acid side chains has been observed only recently (24–26), and thus far the majority of the reports have involved oxidation/reduction of a tyrosine residue. Only in cytochrome-*c* peroxidase has tryptophan been reported to fulfill an electron transport function (27) and, in this enzyme, the magnetic resonance characteristics of the radical are obscured by its interaction with the Fe(III) of the heme chromophore (28). The photoactivation process in photolyase offers an opportunity to characterize tryptophan oxidation in a second system, and here the absence of the metal cofactors indicates that the magnetic resonance properties of the radical are likely to be more straightforward. If this is the case, then, selective isotopic substitution of the tryptophan, which can be achieved in a controlled manner in photolyase, should allow a thorough investigation of the hyperfine interactions in the radical, from which an assessment of the spin density distribution can be made. Our initial magnetic resonance results (16) indicated that such an approach would be successful.

Trp-306 as the Electron Donor. Fig. 1*A* shows a steady-state EPR spectrum of the Enz-$FADH^0$ form of photolyase isolated from a tryptophan auxotroph grown in medium supplemented with tryptophan. The spectrum of Enz-$FADH^0$ from bacteria grown on specifically labeled tryptophan showed line widths and δ values identical to those in Fig. 1*A* (data not shown). Flash photolysis (17-μs flash) of Enz-$FADH^0$ produced an intense transient whose amplitude and sense of signal changed with applied magnetic field. Fig. 1*B* shows an example of such a kinetic trace at the indicated field value relative to the Enz-$FADH^0$ spectrum in Fig. 1*A*. The directions of the transient spikes observed at different field positions were reproducible and were not consistent with the $FADH^0$ spectrum. Therefore, the signals must arise from the light-induced generation of another radical(s) in the g = 2 region. The maximum generation of the light-induced transient required $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ in the photolysis reaction buffer. These reagents reoxidize the reduced flavin so that it does not accumulate during data acquisition.

Using gated integration (48-μs integration window and 4-μs delay following flashlamp trigger) and ac coupling (ν > 10 Hz) to discriminate against stable signals ($FADH^0$), we obtained the spectrum of the transient in Enz-$FADH^0$ induced by the light flash (Fig. 2, spectrum A). The spectrum has emissive/absorptive patterns, which indicates that the radical is born in a spin-polarized state, as discussed in detail elsewhere (ref. 16 and unpublished work), and consists of three well-resolved components with splitting of 15–16 G as indicated by the three solid upward arrows in spectrum A. Superimposed on each of the three resolved components is partially resolved fine structure that reflects an additional coupling of ≈5 G, as indicated by the dashed downward arrows on the low-field, resolved transitions in spectrum A. Upon deuteration of the tryptophan indole ring α protons (tryptophan-d_5), partially resolved hyperfine structure collapses, but the three major components are still observable, as indicated by the solid

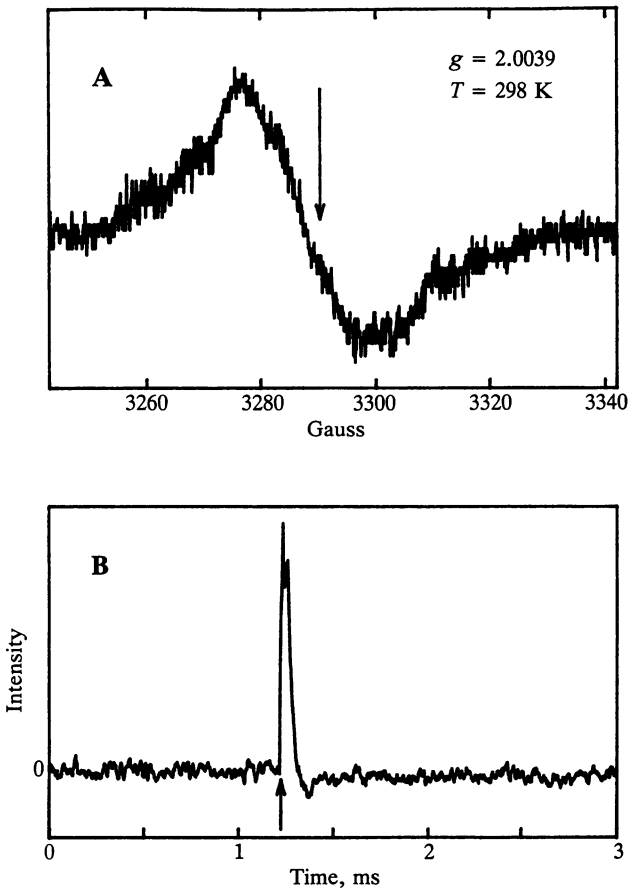

FIG. 1. EPR detection of light-induced radical in *E. coli* DNA photolyase. The dark-stable EPR spectrum of Enz-FADH^0 (*A*) and the kinetic transient of Enz-FADH^0 (*B*) are shown. Arrow in *A* is the field position at which the kinetic transient is taken in each scan. Arrow in *B* is the time at which the lamp was fired in each scan. For the kinetic trace in *B*, 1000 flashes were averaged. Instrument conditions: microwave frequency, 9.22226 GHz; microwave power, 6.3 mW; modulation amplitude, 2.8 G. Enzyme concentration was ≈0.1 mM. All experiments were performed at 298 K in photolysis reaction buffer under a gentle stream of cold nitrogen gas.

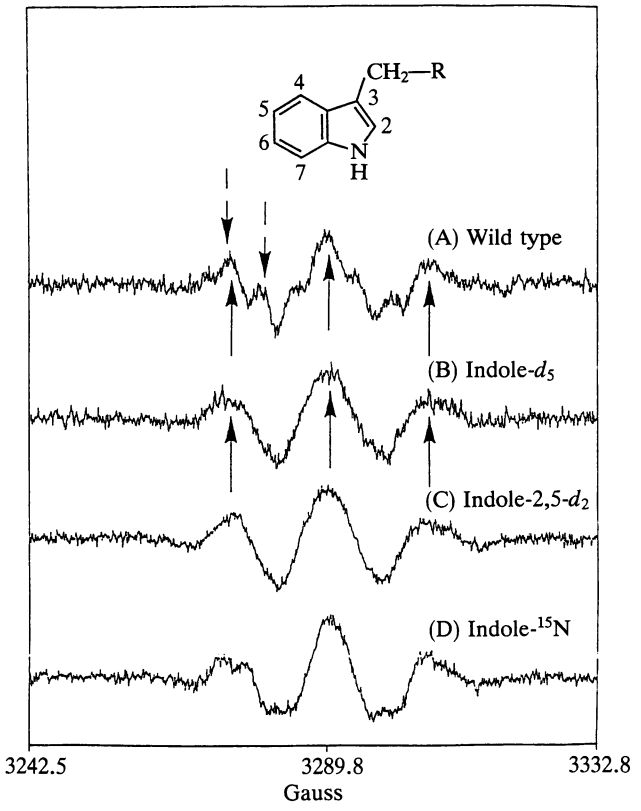

FIG. 2. Time-resolved EPR detection of the light-induced tryptophan radical in *E. coli* DNA photolyase. All spectra are in the first-derivative mode. Spectrum A: Enz-FADH^0 in wild type; 5 scans were averaged to obtain spectrum A. Spectrum B: Enz-FADH^0 labeled with [*indole*-$^2H_5$]tryptophan (indole-d_5); 20 scans were averaged to obtain the spectrum. Spectrum C: Enz-FADH^0 labeled with [*indole*-2,5-$^2H_2$] tryptophan (indole-2,5-d_2); 20 scans were averaged to obtain the spectrum. Spectrum D: Enz-FADH^0 labeled with [*indole*-^{15}N]tryptophan; 20 scans were averaged to obtain the spectrum. The solid upward arrows in spectra A and B show the major coupling, and the dashed downward arrows show the smaller coupling that is superimposed on this large coupling. The numbering scheme for tryptophan is shown at the top. Instrument conditions: microwave frequency, 9.22226 GHz; microwave power, 6.3 mW; modulation amplitude, 2.8 G; time constant, 35 μs. A 4-μs delay and a 48-μs aperture were used for the integration window. Enzyme concentration was ≈0.1 mM.

arrows in spectrum B of Fig. 2, from which we concluded that the radical indeed arose from a tryptophan side chain (16).

To identify the particular residue in *E. coli* photolyase that is responsible for photoreduction of the dark-stable flavin radical, we obtained transient EPR spectra of Trp → Phe mutant photolyases (Fig. 3). All mutant proteins tested gave absorption and steady-state EPR spectra identical to the wild type (data not shown) but behaved differently with regard to the transient EPR spectrum. No transient EPR signal was detected with W306F and W306Y (Fig. 3, spectra B and C). In contrast W157F (Fig. 3, spectrum D) and W418F (spectrum E) had no effect on the transient spectrum. Since chemical reduction of FADH^0 in the W306F and W306Y mutants restores catalytic activity identical to that of wild-type enzyme (15), the absence of transient EPR signal in these mutants is not due to conformational change but is the consequence of eliminating the photoreductant, which these data and the earlier optical data show is Trp-306.

H-Atom or Electron Transfer. The results presented so far, combined with those obtained from laser flash photolysis (13, 14), lead to the general FADH^0 photoreduction scheme presented in Fig. 4. Photoexcitation of FADH^0 generates the excited doublet state of the flavin radical, which then decays by intersystem crossing to yield the quartet state within 100 ps. The long-lived quartet state of FADH^0 (1 μs) is then quenched either by a H-atom transfer, generating the tryptophan neutral radical and $FADH_2$, or by an electron transfer to generate the tryptophan cation radical and $FADH^-$. On the basis of time-resolved absorption studies of FADH^0 reduction, it was tentatively proposed that the reduction of the excited state involved H-atom abstraction from a tryptophan residue (14), whereas flash-induced Pyr<>Pyr repair experiments led to the suggestion that the ground-state flavin was in the $FADH^-$ form, which implies an electron-transfer mechanism (9).

Time-resolved EPR in combination with specifically labeled photolyase can be used to test these two proposals. Molecular orbital (MO) calculations by the Hückel–McLachlan technique have revealed that the spin density of tryptophan radical is localized primarily at the C-2 and C-3 positions in the cation radical and at C-3 and N-1 in the neutral radical (Fig. 5; ref. 29). We confirmed the spin density distribution pattern shown in Fig. 5, for the neutral and cation radicals by carrying out a series of intermediate neglect of differential overlap (INDO) calculations on these species; the INDO-determined spin densities were essentially the same as those reported from the Hückel–McLachlan calculations. Although the MO calculations provide only an approximate representation of the spin density distribution, they are quite

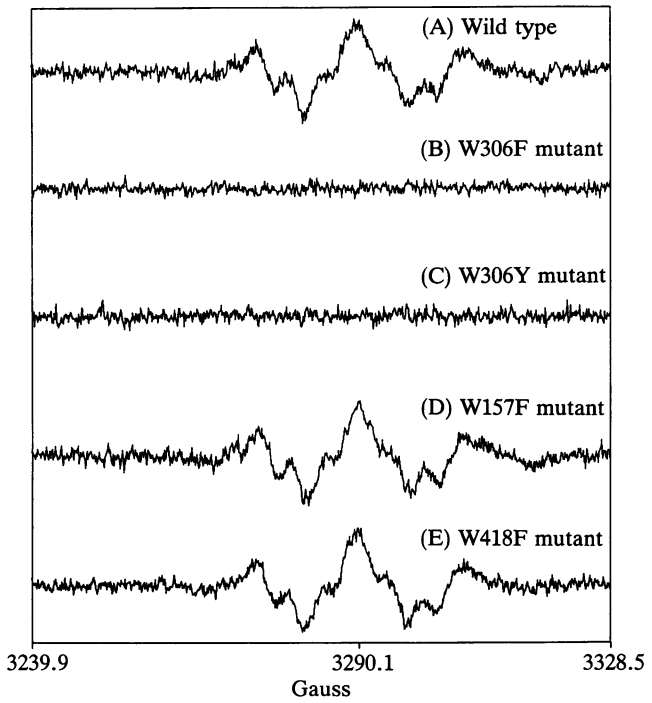

FIG. 3. Effect of Trp → Phe replacement on time-resolved EPR spectrum of the light-induced tryptophan radical in *E. coli* DNA photolyase (Enz-FADH^0). Experimental conditions are the same as in Fig. 2 except that, for all spectra, five scans were averaged to obtain the final spectrum.

reliable in predicting the basic features of the spin density distribution in organic radicals (30–33). The qualitatively different patterns of spin density distribution predicted for the tryptophan radicals (Fig. 5) by the MO calculations indicate that deuteration at the C-2 position will affect the spectrum significantly for the cation radical case, but not for the neutral radical. They also predict that a three line pattern arising from coupling to the C-3 β-methylene protons may be expected in the case of the cation radical, but for the neutral radical a more complicated pattern involving major couplings to both the β-methylene protons and the indole ^{14}N may be expected. At first glance, then, the spectra of the protonated and tryptophan-d_5 radicals are most consistent with a cation radical.

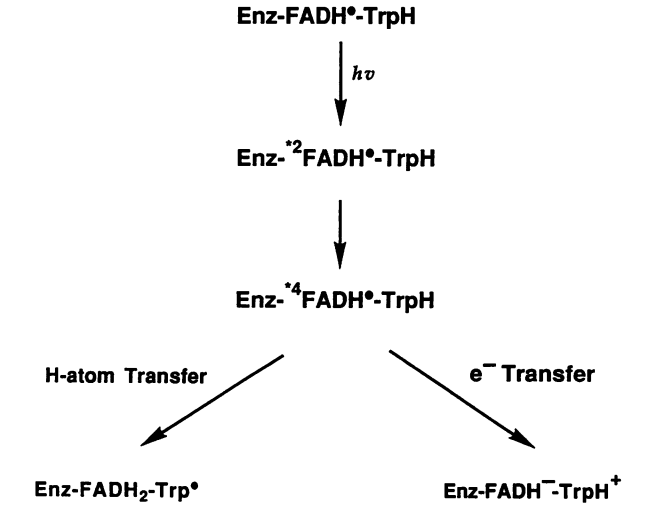

FIG. 4. Two proposed photoreduction mechanisms of E-FADH^0 in DNA photolyase: H-atom transfer vs. electron transfer.

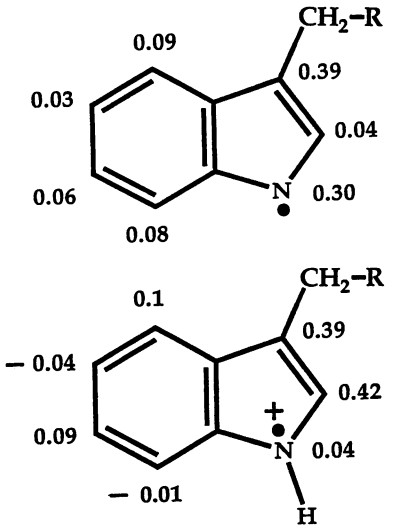

FIG. 5. The spin density distribution in tryptophan neutral (*Upper*) and cation (*Lower*) radicals calculated by the Hückel–McLachlan technique. Values are from ref. 29.

The results of these MO calculations suggest that it is possible to differentiate between the two mechanisms (electron vs. H-atom transfer) experimentally, by examining the transient EPR spectra of the protein samples containing indole-^{15}N, indole-2,5-d_2, and indole-d_5 tryptophans. If the unpaired electron in the transient species were primarily localized on the C-2 and C-3 positions, as is the case for the tryptophan cation radical, the spectrum of tryptophan-2,5-d_2 sample is expected to be equivalent or similar to that of the tryptophan-d_5 sample. Spectra B and C of Fig. 2 show that the two spectra are nearly identical; this observation is consistent with a cation radical species and shows that a significant fraction of the free electron is localized at either C-2 or C-5. Significant spin density at C-5 is not expected, because the MO calculations (Fig. 5) show that the spin density is small for both the cation and the neutral radical at this position. Therefore, we conclude that the coupling to the C-5 position is insignificant and that a large portion of the unpaired electron spin in the radical resides on the C-2 position, which is in agreement with the spin density distribution predicted for the cation radical, but not with that predicted for the neutral radical (Fig. 5). To ascertain whether the ^{14}N nucleus was responsible for the major splitting, as would be expected for a neutral radical, the time-resolved EPR spectrum of ^{15}N-labeled photolyase was taken under the same experimental conditions. This isotope-substitution experiment has the capability to distinguish the cation from the neutral species, as the MO calculations predict an 18-G hyperfine coupling component for N-1 in the neutral species; for the cation radical, the calculations show that the largest nitrogen coupling will be <3 G. From the results of these calculations, the major three-line pattern in the EPR spectrum would arise from the large nitrogen coupling, in the case of the neutral radical, but not if the radical were the cationic species. The spectrum obtained with ^{15}N-labeled sample (Fig. 2, spectrum D) has the same major three-line splitting as the protonated sample (Fig. 2, spectrum A), indicating that the hyperfine splitting contribution of the ^{14}N nucleus to the spectrum is small. The absence of large coupling due to nitrogen further argues against the transient species being a neutral radical. This leads to the conclusion that the major three-line splitting observed with the protonated sample (Fig. 2, spectrum *A*) arises from hyperfine coupling to the two methylene protons at the C-3 position. The results in Fig. 2, which show that the unpaired electron is localized primarily at C-3 and C-2, together with MO calculations summarized in Fig. 5, dem-

onstrate that the transient species associated with the major EPR signal (g = 2.0039) is a tryptophan cation radical that results from electron abstraction by photoexcited $FADH^0$. Therefore, of the two alternative pathways for photoactivation in photolyase shown in Fig. 4, the EPR data provide strong evidence in favor of the electron transfer/tryptophan cation radical route.

Status of Reduced Flavin in Photolyase. Photolyase repairs pyrimidine dimer by electron transfer (9, 10) from flavin to the dimer. However, picosecond flash photolysis of the enzyme–substrate complex did not reveal a transient ascribable to $FADH_2^{+\cdot}$ that was expected if the enzyme contained the reduced flavin in the $FADH_2$ form (9). It was therefore suggested that the two-electron-reduced flavin in photolyase was in the $FADH^-$ form, which would yield $FADH^0$ upon electron transfer to the substrate; the ill-defined transient in the 500- to 700-nm region seen in the picosecond flash photolysis experiment was consistent with $FADH^0$ absorption (9). The results presented in this paper support that assignment. Additional support for this proposal was provided in a recent study by Hartman and Rose (34). Those authors investigated flavin-sensitized splitting of pyrimidine dimers in a model system and, by measuring the solution pH dependence of the dimer splitting efficiency, concluded that the reduced flavin anion ($FMNH^-$) was 8-fold more efficient than neutral flavin ($FMNH_2$) in splitting dimers. Since the excited singlet state of reduced flavin in photolyase repairs pyrimidine dimers with a quantum yield of near unity (35), it is quite likely that this high-efficiency electron transfer is achieved by having the flavin in the $FADH^-$ form. Finally, the absorption spectrum of the fully reduced, enzyme-bound flavin in *E. coli* photolyase (20) shows a close similarity to the spectral properties of protein-bound $FMNH^-$, rather than $FMNH_2$ (36). Thus, we propose that the $FADH^-$ form of flavin in photolyase that arises by photoreduction and is evident on a microsecond time scale does not become protonated at longer times and represents the physiological form of the flavin *in vivo*.

This work was supported by National Institutes of Health Grants GM31082 (A.S.), GM37300 (G.T.B.), and ES05557 (S.-T.K.).

1. Sancar, A. (1992) in *Advances in Electron Transfer Chemistry*, ed. Mariano, P. E. (JAI, London), Vol. 2, pp. 215–272.
2. Sancar, A. & Sancar, G. B. (1984) *J. Mol. Biol.* **172**, 223–227.
3. Eker, A. M. P., Kooiman, P., Hessels, J. K. C. & Yasui, A. (1990) *J. Biol. Chem.* **265**, 8009–8015.
4. Johnson, J. L., Hamm-Alvarez, S., Payne, G., Sancar, G. B., Rajagoplan, K. V. & Sancar, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2046–2050.
5. Eker, A. P. M., Hessels, J. K. C. & Velde, J. V. (1988) *Biochemistry* **27**, 1758–1765.
6. Kiener, A., Husain, I., Sancar, A. & Walsh, C. (1989) *J. Biol. Chem.* **264**, 13880–13887.
7. Kim, S. T., Heelis, P. F., Okamura, T., Hirata, Y., Mataga, N. & Sancar, A. (1991) *Biochemistry* **30**, 11262–11270.
8. Lipman, R. S. A. & Jorns, M. S. (1992) *Biochemistry* **31**, 786–791.
9. Okamura, T., Sancar, A., Heelis, P. F., Begley, T. P., Hirata, Y. & Mataga, N. (1991) *J. Am. Chem. Soc.* **113**, 3143–3145.
10. Kim, S. T., Sancar, A., Essenmacher, C. & Babcock, G. T. (1992) *J. Am. Chem. Soc.* **114**, 4442–4443.
11. Payne, G., Heelis, P. F., Rohrs, B. R. & Sancar, A. (1987) *Biochemistry* **26**, 7121–7127.
12. Li, Y. F. & Sancar, A. (1991) *Nucleic Acids Res.* **18**, 4885–4890.
13. Okamura, T. A., Sancar, A., Heelis, P. F., Hirata, Y. & Mataga, N. (1989) *J. Am. Chem. Soc.* **111**, 5967–5969.
14. Heelis, P. F., Okamura, T. & Sancar, A. (1990) *Biochemistry* **29**, 5694–5698.
15. Li, Y. F., Heelis, P. F. & Sancar, A. (1991) *Biochemistry* **30**, 6322–6329.
16. Essenmacher, C., Kim, S. T., Atamian, M., Babcock, G. T. & Sancar, A. (1993) *J. Am. Chem. Soc.* **115**, 1602–1603.
17. Sancar, G. B., Smith, F. W. & Sancar, A. (1983) *Nucleic Acids Res.* **11**, 677–678.
18. Kim, S. T. & Rose, S. D. (1992) *J. Photochem. Photobiol. B.* **12**, 179–191.
19. Sancar, G. B., Jorns, M. S., Payne, G., Fluke, D. J., Rupert, C. S. & Sancar, A. (1987) *J. Biol. Chem.* **262**, 492–498.
20. Heelis, P. F., Payne, G. & Sancar, A. (1987) *Biochemistry* **26**, 4634–4640.
21. Jorns, M. S., Wang, B. & Jordan, S. P. (1987) *Biochemistry* **26**, 6810–6816.
22. Hoganson, C. W. & Babcock, G. T. (1988) *Biochemistry* **27**, 5848–5855.
23. Malhotra, K., Kim, S. T., Walsh, C. T. & Sancar, A. (1992) *J. Biol. Chem.* **267**, 15406–15411.
24. Sjoberg, B.-M., Reichard, P., Graslund, A. & Ehrenberg, A. (1978) *J. Biol. Chem.* **253**, 6863–6865.
25. Stubbe, J. (1989) *Annu. Rev. Biochem.* **58**, 257–285.
26. Hoganson, C. W. & Babcock, G. T. (1992) *Biochemistry* **31**, 11874–11880.
27. Sivaraja, M., Goodin, D. B., Smith, M. & Hoffman, B. M. (1989) *Science* **245**, 738–740.
28. Barry, B. A. (1993) *Photochem. Photobiol.* **57**, 179–188.
29. Hoffman, B. M., Roberts, J. E., Kang, C. H. & Margoliash, E. (1981) *J. Biol. Chem.* **256**, 6556–6564.
30. McLachlan, A. A. D. (1960) *Mol. Phys.* **3**, 233–252.
31. Streitweiser, A., Jr. (1961) *Molecular Orbital Theory for Organic Chemists* (Wiley, New York).
32. Tople, J. A., Beveridge, D. L. & Dobosh, P. A. (1967) *J. Chem. Phys.* **47**, 2026–2033.
33. Tople, J. A., Beveridge, D. L. & Dobosh, P. A. (1968) *J. Am. Chem. Soc.* **90**, 4201–4209.
34. Hartman, R. F. & Rose, S. D. (1992) *J. Am. Chem. Soc.* **114**, 3559–3560.
35. Kim, S. T. & Sancar, A. (1991) *Biochemistry* **30**, 8623–8630.
36. Ghisla, S., Massey, V., Lhoste, J.-M. & Mayhew, S. G. (1974) *Biochemistry* **13**, 589–597.