# Energy Transfer (Deazaflavin → $FADH_2$) and Electon Transfer ($FADH_2$ → T<>T) Kinetics in *Anacystis nidulans* Photolyase†

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ABSTRACT: DNA photolyases catalyze the photocycloreversion of cyclobutane pyrimidine dimers. The enzyme from the cyanobacterium *Anacystis nidulans* contains two chromophores, 1,5-dihydroflavin adenine dinucleotide ($FADH_2$) and 7,8-didemethyl-8-hydroxy-5-deazariboflavin (8-HDF). The photophysical/photochemical reactions leading to DNA repair were investigated by using time-resolved and steady-state fluorescence spectroscopy. It was found that the excited singlet state of 8-HDF transfers energy to $FADH_2$ at a rate of $1.9 \times 10^{10}$ $s^{-1}$ and a quantum yield of 0.98. Using the Forster equation for long-range energy transfer and assuming random orientations of the donor and acceptor the interchromophore distance was calculated to be 15 Å. The excited singlet $FADH_2$ which forms either by energy transfer from 8-HDF or by direct absorption of a photon has a lifetime of 1.8 ns in the absence of substrate and 0.14 ns in the presence of the photodimer indicating electron transfer from the $FADH_2$ excited singlet state to the dimer at a rate of $6.5 \times 10^9$ $s^{-1}$ and quantum efficiency of 92%.

## INTRODUCTION

The cyclobutadipyrimidine photodimers are produced in DNA by far-UV (200–300 nm) irradiation and repaired by DNA photolyases which utilize near-UV–visible photons (300–500 nm) to initiate the photocycloreversion reaction (Sancar, 1992). Although it has been shown recently that a specific Trp residue in the polypeptide chain (Trp 277 in *Escherichia coli* DNA photolyase) can photosensitize splitting of Pyr<>Pyr[1] by 250–300 nm (Kim et al., 1992b), the vast majority of the photoreactivating photons are absorbed by two noncovalent chromophores.

Photolyases are divided into two classes according to their chromophore compositions. The folate class enzymes contain 1,5-dihydroflavin adenine dinucleotide ($FADH_2$; Sancar & Sancar, 1984) and 5,10-methenyltetrahydrofolate (MTHF; Johnson et al., 1988) while the deazaflavin class enzymes contain $FADH_2$ and 7,8-didemethyl-8-hydroxy-5-deazariboflavin (8-HDF; Eker et al., 1988; 1990). The MTHF chromophore in the folate class, with its high extinction coefficient in the near-UV and visible ranges, functions as a photoantenna, absorbs 350–400-nm photons, and then transfers its excitation energy to $FADH_2$ by singlet–singlet mechanism (Kim et al., 1991; Lipman & Jorns, 1992). The $FADH_2$ in its singlet excited state, then, catalyzes cycloreversion of Pyr<>Pyr by electron transfer to the substrate (Okamura et al., 1991; Kim et al., 1991; 1992a). Indirect evidence suggests that 8-HDF has a function analogous to that of MTHF. Thus, deazaflavin class photolyases expressed in *E. coli* which does not synthesize deazaflavin are functionally competent (Yasui et al., 1988; Takao et al., 1989a,b; Kobayashi et al., 1989) and excitation of 8-HDF causes photoreduction of flavin neutral radical ($FADH^0$) to $FADH_2$ (Eker et al., 1990) in *Anacystis nidulans* photolyase presumably by electronic energy transfer to $FADH^0$ followed by electron transfer from an adjacent amino acid to the excited $FADH^0$. However, model studies of the Pyr<>Pyr photosplitting reaction have shown that 5-deazaflavin (Rokita & Walsh, 1984) and 8-HDF (Eker et al., 1981) are capable of direct photosensitization of the cleavage of the cyclobutane ring of T<>T.

In a recent study to define the roles of the chromophores in deazaflavin class photolyases, we have shown that the E–$FADH_2$ form of *A. nidulans* photolyase is catalytically active but that enzyme-bound 8-HDF cannot catalyze repair in the absence of $FADH_2$ and thus suggested that the sole function of 8-HDF was the sensitization of $FADH_2$ by electronic energy transfer (Malhotra et al., 1992). In this paper using E–8HDF, E–$FADH_2$, E–$FADH^0$–8HDF, and E–$FADH_2$–8HDF forms of *A. nidulans* photolyase and time-resolved (picosecond) and steady-state fluorescence measurements, we investigated the interactions of the chromophores with one another and the DNA substrate. These studies have provided direct evidence for high-efficiency energy transfer from 8-HDF to $FADH_2$ and strong evidence for high-efficiency electron transfer from singlet excited state $FADH_2$ to Pyr<>Pyr.

## MATERIALS AND METHODS

*Enzyme. A. nidulans* photolyase was purified from an *E. coli* strain carrying the cloned *A. nidulans phr* gene by successive chromatography on Blue Sepharose (Sigma), Bio-Gel P-100 (Bio-Rad), and single-strand DNA–cellulose (Sigma) as described previously (Malhotra et al., 1992). Repetitive (3–4 times) passing through the DNA–cellulose resin helped obtain enzyme with greater than 99% purity. Enzyme purified in this manner contained stoichiometric amount of flavin in the $FADH^\circ$ form and no 8-HDF. The radical form of the enzyme was converted to the fully reduced form (E–$FADH_2$) by photoreduction under an oxygen-free argon atmosphere (Heelis et al., 1987). Enzyme containing stoichiometric amounts of both $FADH_2$ and 8-HDF was

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[1] Abbreviations: Pyr<>Pyr, T<>T, cyclobutadipyrimidine photodimers of the indicated compositions; 8-HDF, 7,8-didemethyl-8-hydroxy-5-deazariboflavin; E–8HDF, E–$FADH_2$, etc., photolyase indicated chromophores.

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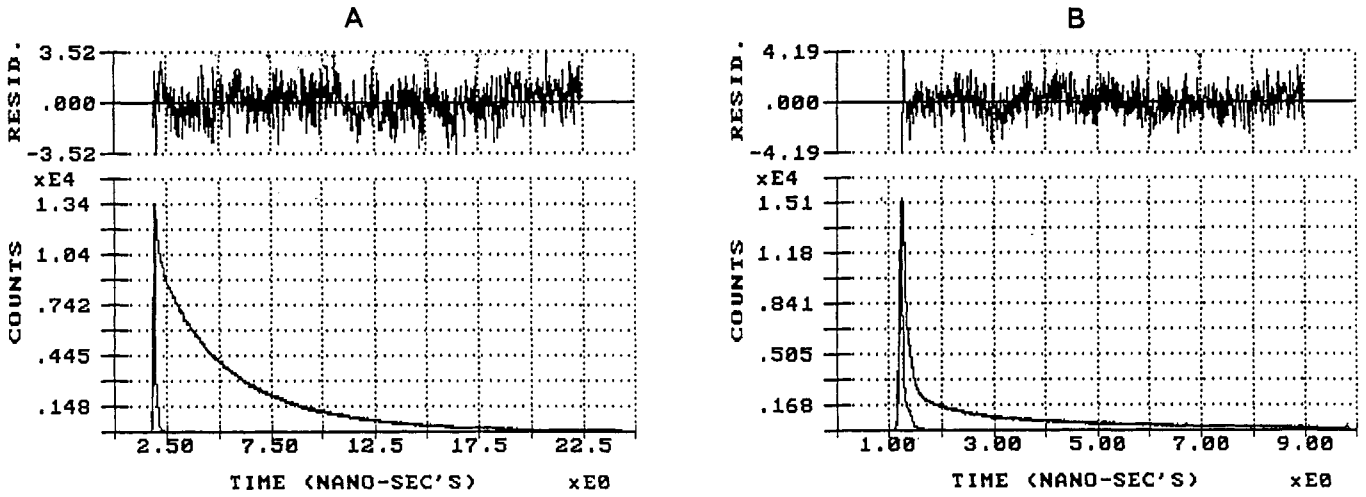


FIGURE 1: Fluorescence decay curves of (A) E–8HDF and (B) E–8HDF–$FADH_2$. The enzyme ($A_{355}$ = 0.2) in 50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 5 mM DTT, 100 mM KCl, and 50% glycerol was excited at 355 nm. Emission was measured at >435 nm.

prepared by supplementing E–$FADH^0$ with 8-HDF followed by photoreduction of the flavin radical with $\lambda > 580$ nm (Payne et al., 1990; Malhotra et al., 1992). Apoenzyme was prepared as described previously (Malhotra et al., 1992), and the E–8HDF and E–$FAD_{ox}$ forms of the enzyme were prepared by incubating with the appropriate cofactor followed by removal of the unbound chromophore by gel filtration and dialysis (Payne et al., 1990).

*Substrate. cis,syn*-T<>T was prepared by photosensitized irradiation of a TpT solution (Kim & Sancar, 1991). The dinucleotide was diluted to 2 mM in 10% aqueous acetone. The solution was deoxygenated by purging with argon and irradiated under argon atmosphere. Photodimerization was accomplished by irradiating with light from a 450-W Hanovia lamp fitted with a Pyrex filter. The reaction was monitored by UV spectroscopy; 100-$\mu$L aliquots were taken and evaporated to dryness in vacuo, the residue was dissolved in water, and absorption spectra were taken. After 4 h of photolysis, ring closure was almost quantitative. To purify the *cis,syn*-T<>T from other minor photoproducts, the entirety of the sample was lyophilized, redissolved in 200 $\mu$L of water, and injected onto Waters $\mu$Bondpack C18 reverse-phase column. The column was developed with a linear gradient of 180 mL of 75 mM $KH_2PO_4$ (pH 4.5) to 180 mL of 40% (v/v) MeOH/75 mM $KH_2PO_4$ (pH 4.5) at 3 mL/min. The purified product was reinjected onto the column and eluted using a water/methanol gradient (0–40%). The fractions were monitored by absorbance and fluorescence spectroscopy. The HPLC instrumentation consisted of two LKB Model 2150 dual-piston pumps, an LKB Model 2152 controller, and a Rhodyne Model 7125 sample injector with a 500-$\mu$L loop. An LKB Model 2151 UV monitor was used for column standardization tests. Reverse-phase HPLC was performed on a Whatman Partisil ODS-3 column with a guard column containing the same packing material.

*Steady-State Fluorescence.* The steady-state fluorescence measurements were made with an SLM 8000 fluorometer with excitation and emission monochromators (Barrow & Lentz, 1983). The polarization ($P$) was obtained by the T-format method from the SLM Instruments. The fluorescence quantum yield of E–8HDF was determined by comparison of the integrated area of the fluorescence emission curve with the corrected emission spectra of two standard solutions. The fluorescence standards used were quinine sulfate dihydrate ($\Phi_F$ = 0.7 in 0.1 N $H_2SO_4$) and riboflavin ($\Phi_F$ = 0.24 in phosphate buffer, pH 7.0).

*Time-Resolved Fluorescence and Flash Photolysis.* Picosecond fluorescence lifetime measurements and laser flash photolysis were performed at the Center for Fast Kinetics Research (CFKR) at the University of Texas, Austin, Texas (Kim et al., 1991). Laser flash photolysis was conducted with a mode-locked Nd-YAG laser as the excitation source to produce 30-ps pulses of 355 nm. The reaction mixture, containing 50 mM Tris-HCl, pH 7.5, 100 mM KCl, 1 mM EDTA, 5 mM dithiothreitol, 50% (v/v) glycerol, and the E–$FADH^0$–8HDF form of the enzyme at a concentration which had an absorbance of ca. 0.3 at the excitation wavelength. The solution was deoxygenated with a gentle stream of cold nitrogen gas. The deoxygenated solution was irradiated with a filtered ($\lambda > 485$ nm) output of a 5000-W Hg–Xe lamp to reduce the E–$FADH^0$–8HDF form of the enzyme to the E–$FADH_2$–8HDF form prior to laser photolysis. The transient differential absorption spectrum reported was obtained by averaging 200 individual laser shots.

## RESULTS

To understand the interactions between the chromophores in the excited state with one another and with the substrate, we investigated the photophysical properties (fluorescence and absorbance) of enzyme containing one or both chromophores, in the absence or presence of substrate.

*Fluorescence Lifetime of 8-HDF Bound to Apoenzyme.* The fluorescence decay of enzyme-bound 8-HDF is shown in Figure 1A. The decay curve could not be fitted to a single exponential but it fit a biexponential reasonably well with $\tau_1$ = 2.0 ns ($A_1$ = 0.9) and $\tau_2$ = 4.5 ns ($A_2$ = 0.1) with $\chi^2$ = 1.3. Since the decay of free 8-HDF under the same experimental conditions shows a single exponential with $\tau = 4.2 \pm 0.5$ ns (data not shown), the short-lived component ($\tau_1$) must arise from the enzyme-bound HDF. That our E–8HDF preparation contained some unbound 8-HDF was not unexpected because this chromophore is not that tightly bound to the enzyme as evidenced by the fact that most deazaflavin class photolyase preparations contain some free 8-HDF [see Eker et al. (1990)].

*Effect of $FADH^0$ and $FADH_2$ on the Fluorescence Lifetime of Enzyme-Bound 8-HDF.* Previously, we reported that the absorption spectrum of E–$FADH_2$–8HDF, especially in the 300–500-nm region, is almost suprimposable with the sum of the spectra of E–8HDF and E–$FADH_2$ and is characterized by the absence of charge transfer bands or hypochromism (Malhotra et al., 1992). The absence of hypochromism and

charge transfer bands suggests that when in ground state $FADH_2$ and 8-HDF do not interact electronically. However, there appeared to be a strong interaction in the excited state because of extensive quenching of the steady-state fluorescence of 8-HDF in the E–$FADH_2$–8HDF form of the enzyme and even more so in the E–$FADH^0$–8HDF form. In this study, we have employed time-resolved spectroscopy in order to quantitatively determine the extent of 8-HDF fluorescence quenching in the presence of either $FADH^0$ or $FADH_2$. A typical decay curve of 8-HDF fluorescence in the E–$FADH_2$–8HDF form of the enzyme is shown in Figure 1B. The most satisfactory fit to the data was obtained with a triexponential model with lifetimes of $\tau_1 = 0.05$ ns ($A_1 = 0.94$), $\tau_2 = 2$ ns ($A_2 = 0.04$), and $\tau_3 = 4.4$ ns ($A_3 = 0.02$) with $\chi^2 = 1.1$. The dominant form $\tau_1 = 0.05$ ns can be assigned to the fluorescence lifetime of 8-HDF in the form of E–$FADH_2$–8HDF. The second (minor) component may arise from either E-8HDF or E-$FADH_2$ both of which are known to have lifetimes around 2.0 ns. The third component is most likely due to free 8-HDF which is present to varying degrees in all enzyme preparations. The 8-HDF in E–$FADH^0$–8HDF was very weakly fluorescent. The decay profile was dominated by a short-lived component ($A_1 = 0.90$) of 20–30-ps lifetime (data not shown) but also exhibited other components of lifetimes of 1.5–2.0 ns ($A_2 = 0.06$) and 4.0–4.5 ns ($A_3 = 0.04$). Thus, the $FADH^0$ quenches 8-HDF fluorescence much more efficiently than the $FADH_2$ form of flavin.

*Spectral Overlap between 8-HDF Emission and Flavin Absorption.* The rapid fluorescence quenching of 8-HDF may be interpreted in terms of singlet–singlet energy transfer from 8-HDF to flavin. We are not able to unambiguously demonstrate energy transfer through measurement of sensitized acceptor emission because the acceptor is nonfluorescent in the case of $FADH^0$ and very weakly fluorescent at wavelengths which overlap with donor emission in the case of $FADH_2$. In support of energy transfer, however, Eker et al. (1990) found that excitation at 437 nm (where 80% of the absorption is due to 8-HDF) had essentially the same efficiency as excitation at 585 nm (where only $FADH^0$ absorbs) in causing flavin photoreduction. It has been shown that flavin photoreduction in photolyases involves an electron abstraction by the quartet excited state of $FADH^0$ (Heelis et al., 1987, 1990) from a nearby Trp residue in the apoenzyme (Li et al., 1991). Thus, the evidence for energy transfer from 8-HDF to $FADH^0$ is relatively strong and it is reasonable to assume that it occurs between 8-HDF and $FADH_2$ as well because of the strong fluorescence quenching by the latter. Regarding the mechanism of energy transfer, we note that there is a significant overlap between the 8-HDF emission and $FADH^0$ absorption spectra (not shown). The overlap between the 8-HDF emission and the $FADH_2$ absorption is small (Figure 2) but sufficient enough to allow Forster-type energy transfer (Forster, 1965).

*Excitation Polarization Spectrum of E–8HDF–$FADH_2$.* Assuming Forster-type energy transfer from 8-HDF to flavin, the efficiency of energy transfer ($\Phi_{eT}$) is readily calculated from the fluorescence lifetimes of E–8HDF, E–$FADH^0$–8HDF, and E–$FADH_2$–8HDF according to

$$\Phi_{eT} = 1 - \tau_2/\tau_1 \quad (1)$$

where $\tau_1$ and $\tau_2$ are the fluorescence lifetimes of the donor (8-HDF) in the absence and presence of acceptor ($FADH_2$ or $FADH^0$), respectively. Substituting the values from $\tau_1$ and $\tau_2$, we obtain efficiencies of energy transfer of 0.98 to $FADH_2$ and >0.99 to $FADH^0$. Under such high-efficiency

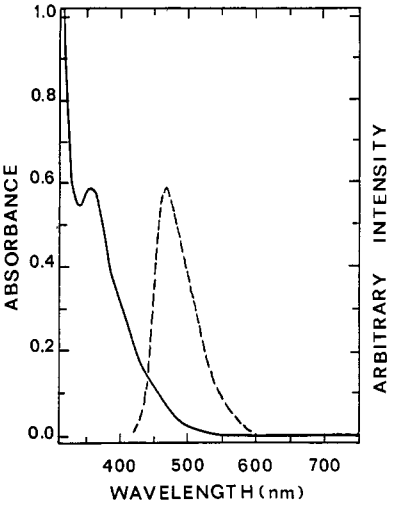


FIGURE 2: Spectral overlap of donor (E–8HDF) emission (broken line) and acceptor (E–$FADH_2$) absorption spectrum (solid line) in *A. nidulans* photolyase. The emission spectrum was measured at an excitation wavelength of 390 nm.

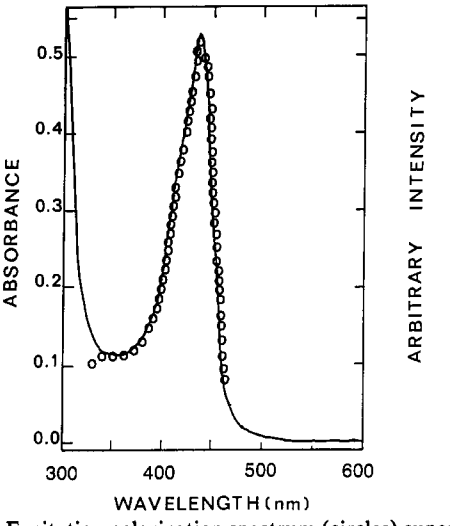


FIGURE 3: Excitation polarization spectrum (circles) superimposed upon the absorption spectrum (solid line) of E–$FADH_2$–8HDF. The fluorescence excitation spectrum was measured at an emission wavelength of 505 nm.

energy transfer conditions, it is expected that the excitation spectrum of E–$FADH_2$–8HDF would correspond to the sum of the absorption spectra of $FADH_2$ and 8-HDF. Figure 3 shows that this is indeed the case. In addition, in agreement with a Forster-type energy transfer, the shape of the excitation spectrum was independent of the emission wavelength monitored.

*Transient Absorption Spectrum of E–$FADH_2$–8HDF.* Assuming energy transfer from 8-HDF to flavin by Forster-type singlet–singlet dipole interaction, in a picosecond flash photolysis experiment one expects to see a difference spectrum dominated by the excited singlet state of 8-HDF at early time points. At longer times the singlet excited state of $FADH_2$ should predominate. Figure 4 shows the transient (difference) spectrum of E–$FADH_2$–8HDF observed 100 ps after a 30-ps laser pulse of 355 nm. This spectrum shows a strong absorption band in the visible region which can be assigned to the first excited singlet state of enzyme-bound $FADH_2$ because of its near-identical shape to the E–$FADH_2$ transient of *E. coli* DNA photolyase (Okamura et al., 1991). In this spectrum we detect no evidence of 8-HDF singlet excited state. Our attempts to obtain the absorption spectrum of E-8HDF excited

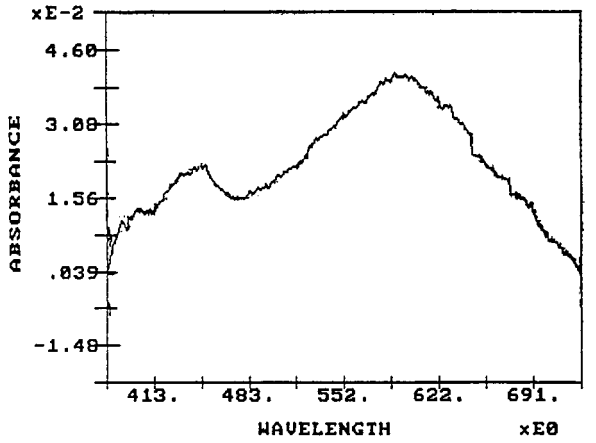


FIGURE 4: Difference absorption spectrum of E–$FADH_2$–8HDF recorded 100 ps after excitation with an average of 200 laser pulses with 10-ps pulse width at a 355-nm excitation wavelength.

states by laser flash photolysis of E-8HDF were unsuccessful because of the instability of this form of the enzyme to intense laser photolysis. Therefore, the assignment of the transient observed in Figure 4 solely to the singlet excited state of $FADH_2$ is not unambiguous. However, on the basis of the short-lifetime ($\tau$ = 50 ps) of 8-HDF in the presence of $FADH_2$, it is quite reasonable to assume that the absorption band corresponds to the first excited singlet state of E–$FADH_2$ with minimal or no contribution from 8-HDF.

*Interchromophore Distance.* The energy transfer efficiency ($\Phi_{eT}$) is related to the donor–acceptor separation ($R$) and the Forster critical distance ($R_0$) by

$$\Phi_{eT} = R_0^6/(R_0^6 + R^6) \quad (2)$$

The value of $R_0$ in angstroms is calculated from

$$R_0 = (9.79 \times 10^3)(JK^2Q_Fn^{-4}) \quad (3)$$

where $n$ is the refractive index of the medium between the donor and the acceptor, $K^2$ is the orientation factor, and $Q_F$ (=0.80) is the fluorescence quantum yield of the donor (8-HDF) in the absence of the acceptor ($FADH_2$ or $FADH^0$). $J$ is the overlap integral between donor emission and acceptor absorption and is calculated (in units of $M^{-1}$ $cm^3$) from

$$J = \int_0^\infty F_D(\lambda)\,\epsilon_A(\lambda)\,\lambda^4\,d\lambda / \int_0^\infty F_D(\lambda)\,d\lambda \quad (4)$$

where $F_D(\lambda)$ is the fluorescence emission of the donor and $\epsilon_A(\lambda)$ is the extinction coefficient of the acceptor. By integrating at 2-nm intervals we obtained the $J$ values of $3.6 \times 10^{-15}$ and $2.05 \times 10^{-14}$ $M^{-1}$ $cm^3$ for the 8-HDF and $FADH_2$ pair and the 8-HDF and $FADH^0$ pair, respectively.

To calculate $R_0$, we assumed $n$ = 1.36 which is the value of 50% glycerol in water (v/v). This value may not be perfectly appropriate since transfer may occur through hydrophobic regions of the active site of the enzyme. However, the values of $n$ change only slightly from one medium to another and as a consequence $R_0$ is not that sensitive to changes in the transfer medium. $K^2$ was assumed to be 2/3, which is the value for a system showing rapid isotropic motion. Substituting the values of $K^2$, $Q_F$, $n^{-4}$, and $J$, we obtain the interchromophore (8-HDF to $FADH_2$) distance of 15 Å.

In this calculation, the most uncertain factor is $K^2$ which can range from 0 to 4 depending on the angle between the dipole moment of the donor's emission transition dipole and the acceptor's absorption transition dipole. To validate the use of $K^2$ = 2/3 in the calculation, the emission anisotropy, $r$, of both the acceptor ($FADH_2$) and the donor (8-HDF) were measured. We found $r$ = 0.15 for E–$FADH_2$ and $r$ = 0.18 for E–8HDF and 298 K. These low values are indicative of considerable rotational freedom for both the donor and the acceptor during energy transfer. If the anisotropies of the donor and the acceptor are low (<0.2), the error in calculated distance is not likely to exceed ±10% of the actual value (Haas et al., 1978; Lakowicz, 1983). Furthermore, we found that E–$FADH_2$–8HDF has $r$ = 0.14 at the excitation wavelength of 440 nm where >99% of absorption is due to 8-HDF. This result also suggests that the transition moments are not parallel ($K^2$ = 4) since the anisotropy characteristic of the 8-HDF is lost in the holoenzyme. If the dipoles were perpendicular ($K^2$ = 0), the $R$ value would be less than 10 Å which would mean orbital overlap and exchange interaction between the two chromophores. However, neither the ground nor the excited-state spectra of E–$FADH_2$–8HDF give any evidence for such interactions. The absence of an exchange-type mechanism is also evident from the fact that the action spectrum of E–$FADH_2$–8HDF is superimposable onto the absorption spectrum of the enzyme (Eker et al., 1990; Malhotra et al., 1992). In light of all of these experimental data, we conclude that the use of $^2/_3$ for $K^2$ does not introduce a significant error and that the value of 15 Å is quite close to the actual interchromophore distance.

*Effect of T<>T on the Fluorescence Lifetime of E–$FADH_2$.* The E–$FADH_2$ form of *A. nidulans* photolyase has fluorescence properties similar to this form of the *E. coli* enzyme. The fluorescence decay curve (data not shown) gives an acceptable fit ($\chi^2$ < 1.4) with two exponential components. The dominant one has a lifetime of 1.8 ns ($A_1$ = 0.9), while the minor component has a lifetime of 3.5 ns ($A_2$ = 0.1). We ascribe the major component to the enzyme-bound $FADH_2$ (which has a lifetime of 1.4 ns in the *E. coli* photolyase; Kim et al., 1991) and the minor component (which is not susceptible to photoreduction, typically observed with enzyme-bound flavin) is ascribed to $FAD_{ox}$ released from the enzyme.

As with the *E. coli* enzyme, in the presence of saturating amount of T<>T there was a drastic decrease in the fluorescence lifetime of *A. nidulans* E–$FADH_2$. The decay profile (data not shown) fits a three-component curve with $\tau_1$ = 0.14 ns ($A_1$ = 0.6), $\tau_2$ = 1.7 ns ($A_2$ = 0.3), and $\tau_3$ = 3.5 ns ($A_3$ = 0.1). The short-lived component ($\tau_1$ = 0.14 ns) was interpreted to be the lifetime of E–$FADH_2$ that is bound to the substrate. Since the repair rate during data collection is expected to be high (Kim & Sancar, 1991), the 1.7-ns component simply reflects the time-averaged amount of free enzyme. Thus, the extent of fluorescence quenching by substrate (from 1.4 ns to 0.14 ns) is similar to that of *E. coli* photolyase (from 1.6 ns to 0.16 ns; Kim et al., 1991). On the basis of these lifetime measurements, the rate of electron transfer during repair by the *A. nidulans* photolyase is calculated from

$$k_{eT} = 1/\tau_1 - 1/\tau_2 = 6.5 \times 10^9\ s^{-1} \quad (5)$$

The quantum yield of electron transfer would then be

$$\Phi_{eT} = k_{eT}\tau_1 = 0.92 \quad (6)$$

Thus, the data obtained from time-resolved spectroscopy are in good agreement with the high quantum yield of repair obtained by steady-state repair measurements (Eker et al., 1990; Malhotra et al., 1992) and provide more definitive evidence for the roles proposed for the two chromophores.

## CONCLUSION

Since the discovery of $FADH_2$ in deazaflavin class photolyases (Eker et al., 1988), it was suspected that 8-HDF may

Table I: Fluorescence Lifetime and Polarization of 8-HDF and $FADH_2$[a]

| chromophore | lifetime (ns) | anisotropy (r) |
|---|---|---|
| 8-HDF | 4.5 | 0.03 |
| E–8HDF | 2.0 | 0.18 |
| E–HDF–8FADH$^0$ | <0.03 | ND |
| E–8HDF–$FADH_2$ | 0.05 | 0.14 |
| E–$FADH_2$ | 1.8 | 0.15 |
| E–$FADH_2$ + T<>T | 0.14 | ND |

[a] For the solution containing 8-HDF, E–8HDF, and E–$FADH_2$–8HDF, the excitation wavelength was 440 nm. For the solution containing E–$FADH_2$, the excitation wavelength was 355 nm.

Table II: Forster-Type Energy Transfer from 8-HDF to $FADH_2$

| parameter[a] | value |
|---|---|
| $k_{eT}$ ($s^{-1}$) | $1.9 \times 10^{10}$ |
| $\Phi_{eT}$ (%) | 98 |
| $R_o$ (Å) | 27 |
| $J$ ($M^{-1}$ $cm^3$) | $3.6 \times 10^{-15}$ |
| $R$ (Å) | 15 |

[a] Parameters: $k_{eT}$ = rate of energy transfer; $\Phi_{eT}$ = quantum efficiency of energy transfer; $R_o$ = Forster's critical distance assuming an orientation factor $K^2 = 0.67$; $J$ = spectral overlap integral; $R$ = calculated distance. Fluorescence quantum yield of donor $\Phi = 0.80$ and refractive index of medium $n = 1.39$ were used for the calculation.

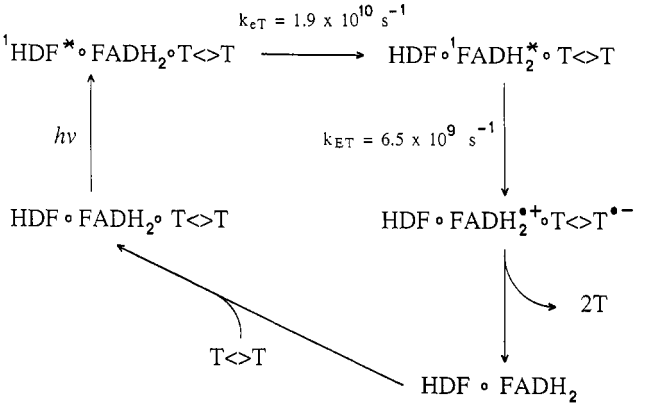


FIGURE 5: Energy and electron transfer kinetics in *A. nidulans* photolyase. The comparison of solar radiation spectrum with the absorption spectra of E–$FADH_2$ and E–$FADH_2$–HDF from *A. nidulans* photolyase reveals that more than 99% of solar light energy is absorbed by 8-HDF.

function as a photoantenna in a manner analogous to that of MTHF in the folate class photolyases (Sancar & Sancar, 1988). However, in contrast with MTHF, 8-HDF is capable of photoinduced redox reactions (Walsh, 1986) which raised the possibility that 8-HDF might directly particpate in catalysis. In this paper, we have shown that the fluorescence of enzyme containing 8-HDF alone is not quenched by substrate because of lack of binding. In enzyme containing both 8-HDF and flavin (in $FADH^0$ or $FADH_2$ form), there is near complete quenching of 8-HDF fluorescence by the flavin consistent with energy transfer from 8-HDF to flavin. By using picosecond fluorescence spectroscopy, we were able to measure the rate of energy transfer between the two chromophores. Similarly, by using fluorescence lifetime measurements of enzyme containing $FADH_2$ only, we observed very efficient quenching by the substrate indicating repair (by electron transfer) at high quantum yield. The photophysical properties of *A. nidulans* photolyase are summarized in Tables I and II, and a minimal kinetic scheme is presented in Figure 5. Many of the properties of this enzyme are similar to those of the folate class *E. coli* DNA photolyase (Kim et al., 1991). However, the efficiency of energy transfer from 8-HDF to $FADH_2$ is 98% compared to 63% efficiency from MTHF to $FADH_2$ in *E. coli* DNA photolyase. As a result, the overall quantum yield of repair by *A. nidulans* enzymes (~0.9) is higher than that of the *E. coli* enzyme (~0.6) even though the singlet excited-state $FADH_2$ of both enzymes transfers electron to the photodimer with identical efficiency (~0.9).

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**Registry No.** $FADH_2$, 1910-41-4; 8-HDF, 37333-48-5; $FADH^\circ$, 81138-30-9; DNA photolyase, 37290-70-3.