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Supporting Information

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Supporting Information

for

Very Fast Product Release and Catalytic Turnover of DNA Photolyase

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Contains details on:
- sample preparation and standard measurements
- experimental setups
- data analysis (including Figure S1)

Sample Preparation and Standard Measurements

Anacystis nidulans photolyase was overproduced in *E. coli* (M. Takao, A. Oikawa, A. P. M. Eker, A. Yasui, *Photochem. Photobiol.* **1989**, *50*, 633–637) and purified as described by Eker et al. (A. P. M. Eker, P. Kooiman, J. K. C. Hessels, A. Yasui, *J. Biol. Chem.* **1990**, *265*, 8009–8015). The purified enzyme was devoid of the antenna chromophore and contained a mixture of two redox states of the flavin adenine dinucleotide (FAD) cofactor: the neutral radical $FADH^{\circ}$ (major form) and the fully oxidized FAD_{ox} (minor form). It was stored at -80°C in the presence of 2-mercaptoethanol and 20% glycerol. Immediately before measurements, the photolyase storage buffer was exchanged against repair buffer (100 mM NaCl, 10 mM phosphate buffer, pH 7.0) with polyacrylamide chromatography microcolumns at 4°C.

For anaerobic photoreduction of $FADH^{\circ}$ to catalytically active $FADH^{-}$, samples containing 10-20 µM photolyase and 10 mM cystein in repair buffer were prepared under nitrogen in a glove box. Photoreduction was carried out on ice with continuous light from a LQX1800 xenon lamp (Linos) suitably filtered (> 500 nm) to avoid photoreduction of oxidized FAD and was monitored by the decrease of the 580 nm absorption of $FADH^{\circ}$.

The substrate was obtained by irradiating short single strands of DNA made of 18 thymines ($(dT)_{18}$) with UV light (312±15 nm) in the presence of acetone (M. M. van Oers, M. H. Lampen, M. I. Bajek, J. M. Vlak, A. P. M. Eker, *DNA Repair* **2008**, *7*, 1309-1318). It contained on average six cyclobutane pyrimidine dimers (CPDs) per strand, as estimated from the 265 nm absorption increase upon complete photorepair assuming $\Delta\varepsilon_{265}$=17 $mM^{-1}cm^{-1}$ per CPD repaired. During UV irradiation, a very minor fraction of the thymines is converted into the (6-4) photoproduct with absorption up to 360 nm and a large fluorescence band centered around 400 nm and extending to >500 nm (W. A. Franklin, K. M. Lo, and W. A. Haseltine, *J. Biol. Chem.* **1982**, *257*, 13 535-13 543). In order to eliminate this parasitic fluorescence, the substrate was treated prior to use with 355 nm laser pulses which upon absorption transform the (6-4) photoproduct into the non-fluorescent Dewar isomer (Smith, C. A. & Taylor, J.-S. *J. Biol. Chem.* **1993**, *268*, 11143-51). Addition of substrate to photoreduced enzyme was done in the glove box. Under such conditions, we observed less than 5 % re-oxidation of photolyase-bound $FADH^-$ after one hour, which was the typical duration of our experiments.

Steady state spectra were recorded with a Cary Eclipse fluorimeter (Varian) and an Uvikon XS spectrophotometer (Secomam).

Experimental Setups

Two-pulse "pump-probe" fluorescence experiments (main Figure 1): The sample was contained in an anaerobic 4x10 mm quartz cell kept at 10 °C. Pump pulses of 5 ns duration and ~90 mJ/cm^2 energy at 355 nm were provided along the 4 mm path by a tripled Brilliant B Nd:YAG laser (Quantel, France). These pulses were polarized circularly by passing through a quarter-wave plate in order to decrease the fraction of photolyases that are not excited because of an unfavorable orientation of their $FADH^-$ transition moment with respect to polarization of the pump beam (M.-L. Groot, R. van Grondelle, J.-A. Leegwater, F. van Mourik, *J. Phys. Chem. B* **1997**, *101*, 7869). Based on an extinction coefficient of ~7000 M^{-1} cm^{-1}, for $FADH^-$ at 355 nm, ~90% of the reduced photolyases should absorb at least one pump photon. Weak and short probe pulses (100 ps, 250 $\mu J/cm^2$ at 355 nm) provided by a Leopard tripled Nd:YAG laser (Continuum) were electronically delayed with respect to the pump pulses and probed the sample fluorescence. The pump and probe beams formed a small angle

and were spatially overlapped in the sample. The sample fluorescence was detected at 90° to the incident laser beams by a R2566U-11 p hotomultiplier tube (Hamamatsu) coupled to a DSO 81004B digital oscilloscope (Agilent). The response of this detection system to the probe pulse was characterized by an instrument response function (IRF) of 325 ps FWHM (dashed line in Figure S1). The detection wavelength was set to 580 nm by means of a 10 nm bandwidth interference filter placed in front of the detector. This wavelength was chosen to minimize the contribution from excited-state solvation processes that were reported to occur essentially below 550 nm (Y.-T. Kao, C. Saxena, L. Wang, A. Sancar, D. Zhong, *Proc. Nat. Acad. Sci. USA* **2005**, *102*, 16128-16132).

Photorepair measurements with continuous light (main Figure 3): The sample was contained in an anaerobic 2x10 mm quartz cell kept at 10 °C. Photorepair was induced by the 350 and 363.8 nm UV lines of a continuous wave Sabre Innova Ar^+ laser (Coherent) that were focused on a pinhole of 0.8 mm diameter in front of the narrow window of the cell, resulting in a light flux of 120 W/cm^2 at the entrance of the cell. The exposition time was controlled by a VS25-S2-S0 Uniblitz shutter (Vincent Associates). Photorepair as a function of exposition time was quantified after sample homogenization by measuring over 2 mm optical path the absorbance increase at 265 nm due to CPD repair induced by several identical pulses (in order to accumulate sufficiently large absorption changes) and recalculation to the volume illuminated by the Ar^+ laser repair beam.

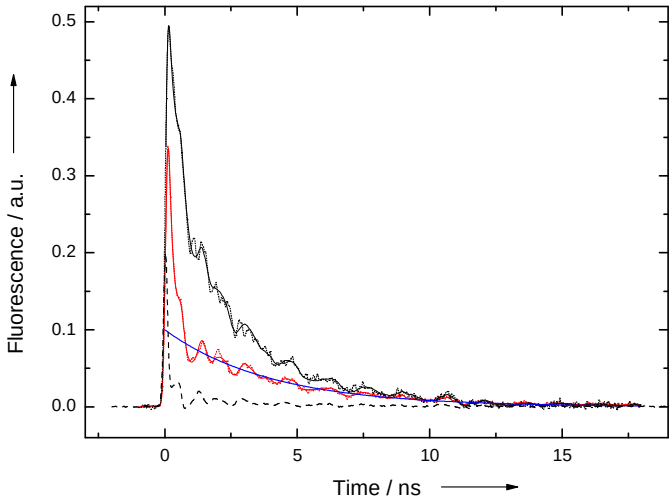

Figure S1: Fluorescence decays of reduced DNA photolyase at 580 nm in the absence of a pump pulse. Black: photolyase without substrate, Red: 280 µM CPD added. Dotted: Data, Solid: Fits (see text), Dashed: IRF

Data Analysis

Steady state fluorescence spectra (inset of Figure 1): For quantifying the quenching of $FADH^-$ fluorescence by the presence of substrate, measured fluorescence spectra were corrected for contributions from oxidized FAD, from a small amount of remaining (6-4) photoproduct, and from impurities in the buffer. The approach relied on the measurement of five raw spectra: buffer ($I^{buffer}(\lambda)$), substrate ($I^{substrate}(\lambda)$), photolyase before reduction ($I^{noRed,noS}(\lambda)$), photolyase after reduction ($I^{Red,noS}(\lambda)$) and reduced photolyase with substrate ($I^{Red,S}(\lambda)$). Corrected spectra for free ($I^{free}(\lambda)$) and bound ($I^{bound}(\lambda)$) photolyase were obtained as follows (s accounts for the substrate dilution):

$$I^{free}(\lambda) = I^{Red,noS}(\lambda) - I^{noRed,noS}(\lambda),$$

$$I^{bound}(\lambda) = I^{Red,S}(\lambda) - I^{noRed,noS}(\lambda) - s \cdot (I^{substrate}(\lambda) - I^{buffer}(\lambda))$$

Note, $FADH^\circ$, the major form of the flavin in isolated photolyase, is silent in steady-state fluorescence.

Time-resolved fluorescence at 580 nm (main panel of Figure 1): A very slow component (lifetime $\tau \approx 4$ ns) was observed with virtually identical amplitude in all our samples (including photolyase before reduction). Most likely, this component originates

from the minor fraction of photolyases containing oxidized FAD. The fluorescence kinetics shown in the main panel of Figure 1 have been corrected for this contribution by subtracting the same amount (0.095 a.u.) of a 4 ns decay (blue line in Figure S1).

The lifetimes of the $FADH^-$ fluorescence of the samples without substrate (black trace) and with substrate (red trace) were first determined by fitting a mono-exponential decay to the data, starting the fit at 200 ps after time zero (defined as the peak of the IRF); the resulting lifetimes were 1.29 ns and 222 ps, respectively (Figure 1).

Alternatively, we used a homewritten reconvolution least square fit software (fit34af4b by Thomas Gustavsson, Laboratoire Francis Perrin, CEA Saclay) and the experimentally determined IRF for biexponential fits of the uncorrected fluorescence traces (Figure S1). The red curve (with substrate) was best fitted with 92% of a 142 ps phase and 8% of a 2.26 ns phase, whereas the black curve (without substrate) was best fitted with 60% of a 230 ps phase and 40% of a 1.17 ns phase, respectively (solid lines).

A previous highly resolved fluorescence study reported a similar bi-exponential decay (20 % 200 ps, 80 % 1.3 ns) at 550 nm for photolyase from *E. coli* depleted of the antenna chromophore (Y.-T. Kao, C. Saxena, L. Wang, A. Sancar, D. Zhong, *Proc. Nat. Acad. Sci. USA* **2005**, *102*, 16 128-16 132). We cannot exclude that the more pronounced fast phase obtained from fitting our data is a convolution artifact, as the IRF was measured with scattered light at 355 nm, whereas fluorescence was detected at 580 nm. Oxidizeed FAD might contribute to the fast phase as well (Y.-T. Kao, C. Saxena, T.-F. He, L. Guo, L. Wang, A. Sancar, D. Zhong, *J. Am. Chem. Soc.* **2008**, *130*, 13 132). Because of these ambiguities, we analyzed the fluorescence traces $I^{S,F}(T,t)$ induced by a weak probe flash given at various times T after a strong repair (pump) flash by decomposing them into a weighted sum of the traces measured without preflash in the presence ($I^{S,noF}(t)$) and in the absence ($I^{noS,noF}(t)$) of substrate, respectively: $I^{S,F}(T,t) = a(T)\ I^{noS,noF}(t) + (1-a(T))\ I^{S,noF}(t)$. The fit parameter a(T) represents the fraction of photolyases showing “non-quenched” fluorescence (plotted vs. *T* in Figure 2). This procedure turned out to be more robust than multi-exponential fits. Note that the contribution from FAD_{ox}, which is present in all measured fluorescence decays, is eliminated automatically by this procedure.