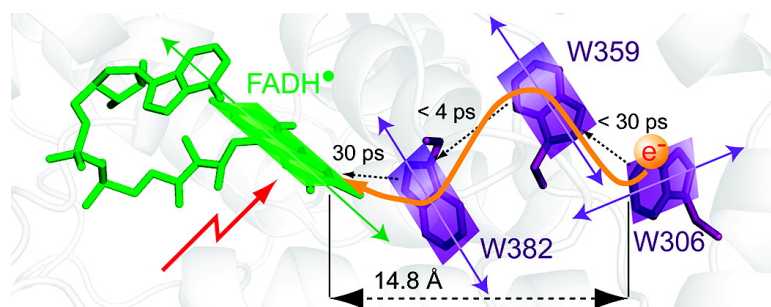Communication

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Electron Hopping through the 15 Å Triple Tryptophan Molecular Wire in DNA Photolyase Occurs within 30 ps

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Long-range electron transport plays a key role in many biochemical processes. Whereas the direct electron transfer (ET) between redox partners becomes too slow to be physiologically relevant at distances greater than ca. 14 Å,[1] efficient long-range transport can take place via hopping mechanisms involving intermediates. Examples include ET through, effectively "one-dimensional", DNA[2,3] and peptide[4] chains. In proteins, multistep ET also takes place in many processes through well-defined intermediates defining a path through the three-dimensional structure. For instance, the presence of a single Trp residue between a Cu center and a chemically attached photoreducible dye in azurin was shown to speed up long-range ET by 2 orders of magnitude to the nanosecond regime.[5] In some cases a range of identical redox-active components bridges the ultimate donor and acceptor. The best-studied example of this case is the triple tryptophan (Trp) chain in DNA photolyase[6−8] (PL). Here, electron transport from a solvent-exposed Trp to a flavin radical (FADH$^\bullet$) located on the other side of the protein is mediated by two intermediate Trp residues. This process is initiated by formation of the excited-state of the flavin radical, and leads to the build-up of the catalytically active fully reduced FADH$^-$ form in ~30 ps.[7] The final FADH$^-$Trp$^{\bullet+}$ charge pair (distance 14.8 Å) is stabilized by deprotonation of the solvent-exposed Trp in 200 ns.[7]

The fact that the trans-protein electron transport along this chain is initiated by light absorption makes it a priori an ideal system to dissect by time-resolved spectroscopy. Complications arise from the fact that the three involved Trp residues are chemically identical and cannot be easily distinguished spectroscopically. The reactions between the Trps have been studied theoretically,[9,10] and experimentally in proteins in which the Trps were altered, one by one, to redox-inert phenylalanine.[11−14] Figure 1 shows the electron transport chain in the *Escherichia coli* enzyme.[15] The distances between the constituents are short enough (3.9−5.2 Å) to enable in principle ET rates as fast as 10^{12} s^{-1},[1,16] depending on the activation barriers. The experimental studies demonstrated that W$_{382}$ is the primary electron donor.[11] However, it cannot be significantly populated in the oxidized state, because it is reduced in <4 ps by W$_{359}$;[14] an upper limit of 10 ns was determined for subsequent W$_{306}$ oxidation.[13] The intriguing question thus arises whether the primary charge separation is rate-limiting and charge could actually transfer to the surface-exposed residue W$_{306}$ within 30 ps, in which case the chain can actually be regarded effectively as a trans-protein "wire". Here, we answer this question using polarized femtosecond spectroscopy to distinguish W$_{359}$ and W$_{306}$.

In W$_{306}$F mutant PL, the isotropic transient spectrum after a few ps (Figure 2A) is very similar to WT, W$_{382}$F and W$_{359}$F PL,[11,14] and corresponds to formation of the excited-state FADH$^{\bullet}$*, with characteristic features that have been discussed previously.[17] Subsequently
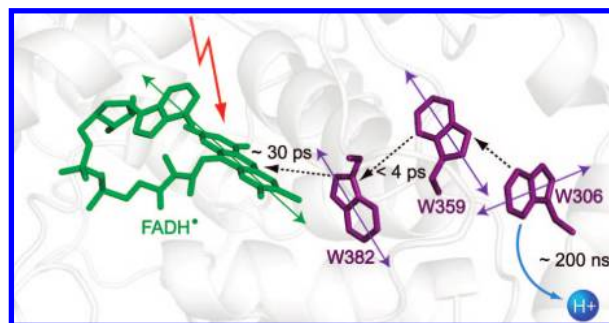
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Figure 1. Disposition of the FADH$^{\bullet}$−W$_{382}$−W$_{359}$−W$_{306}$ chain in DNA photolyase from *E. coli* (PDB entry 1DNP[15]) and scheme of electron and proton transfer reactions. The indicated long axes highlight the different orientation of the ring systems.

single-exponential decay to an end level occurs with a time constant of 27 ps (Figure 2B). This decay time is very similar to those in WT and W$_{359}$F PL,[11,14,18] in both of which the primary donor W$_{382}$ is also present, in agreement with the rate being determined by the initial FADH$^{\bullet}$*W$_{382}$→FADH$^-$W$_{382}$$^{\bullet+}$ charge separation reaction.[14]

The asymptotic spectrum reached after the 27-ps phase under isotropic conditions in the W$_{306}$F protein has a very similar shape and
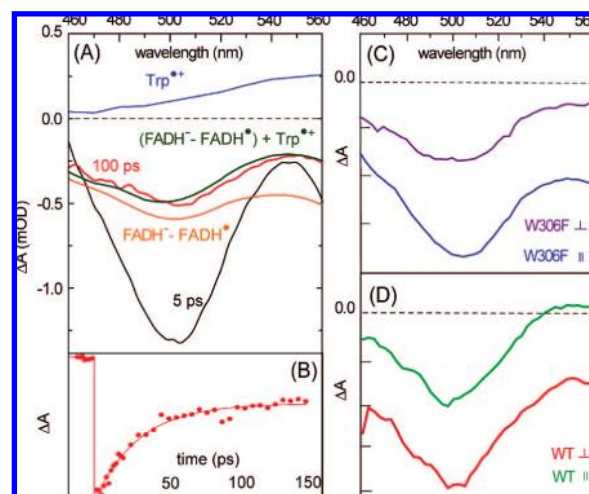

Figure 2. Transient spectra (A) and kinetics at 500 nm (B) of W$_{306}$F PL under isotropic conditions (magic angle between pump and probe polarizations). The 100-ps spectrum is very similar to the corresponding WT spectrum and can be modeled as the sum of the (FADH$^-$−FADH$^{\bullet}$) and the Trp$^{\bullet+}$ spectrum. Expression and purification of *E. coli* PLs,[12] sample preparation[14] and multicolor femtosecond spectroscopy[14] with excitation pulses centered at 620 nm, was as described. Samples were chilled to 8−10 °C. Asymptotic spectra $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ on the 100-ps time scale obtained by global fit of transient spectra with pump and probe pulses polarized parallel and perpendicular for W$_{306}$F (C) and WT (D) PL.

amplitude as in WT,[14] and can be well modeled as the sum of the $(FADH^- - FADH^\bullet)$ and the $Trp^{\bullet+}$ spectrum (neutral Trp does not absorb in the visible) (Figure 2A[8]). We conclude that the rate and yield of initial $FADH^- Trp^{\bullet+}$ formation are independent of the presence of the terminal electron donor W_{306}. As the $FADH^- W_{382}^{\bullet+}$ state is instable and recombines within a few ps,[14] in the $W_{306}F$ mutant protein the long-lived state must be $FADH^- W_{359}^{\bullet+}$. Thus, reduction of $W_{382}^{\bullet+}$ by W_{359} stabilizes the charge separation. At variance with theoretical prediction,[10] this implies that this reaction is downhill.

In the $W_{306}F$ protein, at $t = 10$ ns, the formed Trp radical, presumably $W_{359}^{\bullet+}$, is deprotonated by a protein constituent.[12] The spectrum of this form (maximum ∼500 nm) is quite different and cannot describe the asymptotic spectrum observed on the picosecond time scale. In addition, at $t = 10$ ns the yield of this state was found to be ∼3.5-fold lower than the yield of $FADH^- Trp^{\bullet+}$ in WT,[12] whereas we have now shown that the yields of $FADH^- Trp^{\bullet+}$ are similar at 150 ps. Taken together, our results indicate that charge recombination from the state $FADH^- W_{359}^{\bullet+}$ and $W_{359}^{\bullet+}$ deprotonation both take place on the time scale of 200 ps−10 ns, the latter with a ∼3.5 fold lower rate.

In WT PL it cannot be deduced from our isotropic data whether the radical is located on W_{359} or on W_{306}. The orientation of these Trps in the protein is quite different (Figure 1) and therefore they can be discriminated in principle by determining the angle β between the optical transitions of the excited flavin and the Trp radical using polarization photoselection experiments. The $W_{306}F$ mutant protein serves as a reference for the state $FADH^- W_{359}^{\bullet+}$. Whereas the isotropic asymptotic spectra at 100 ps are very similar for both WT and $W_{306}F$ PL, the corresponding polarized spectra $\Delta A_{||}$ and $\Delta A_{\perp}$ are not (Figures 2C,D), implying that different Trp radicals are involved. The data were quantitatively analyzed (Figure 3) following a procedure described in detail in ref 13. The analysis was limited to the region >500 nm to avoid complications due to contributions of $FADH^-$ absorption at lower wavelengths.

For WT PL, the best correspondence of the experimental and model anisotropy spectra is obtained for $\beta = 50-70°$, and for $W_{306}F$ PL $\beta = 25-30°$. These values are close to the respective values deduced for $t = 10$ ns of ∼75° for WT PL and ∼35° for $W_{306}F$ PL (the latter value corresponds to the angle between the lowest $FADH^\bullet$ transition and the transition of $Trp_{deprot}^\bullet$).[13] The deduced values are also in reasonable agreement with estimations of 79° and 35° of the corresponding angles[13] based on structural data. Altogether, our data clearly demonstrate that in WT PL, after the ∼30 ps phase, it is the surface-exposed W_{306} residue that carries the positive charge. In principle, the charges can be equilibrated between W_{359}
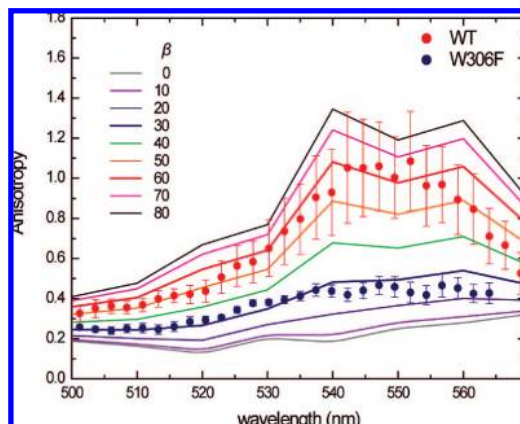
and W_{306} at this stage, but apart from the structural arguments, the above-discussed charge recombination from $FADH^- W_{359}^{\bullet+}$ excludes significant long-lived $W_{359}^{\bullet+}$ build-up. Thus, stabilization of the $FADH^- W_{306}^{\bullet+}$ charge pair by ET from W_{306} via W_{359} to W_{382} occurs in less than 30 ps, and the entire process is rate-limited by the initial $FADH^{\bullet*} W_{382} \rightarrow FADH^- W_{382}^{\bullet+}$ charge separation process.

$W_{359}^{\bullet+} \rightarrow W_{382}^{\bullet+}$ transfer occurs in <4 ps.[14] The present work shows that $W_{306}^{\bullet+} \rightarrow W_{359}^{\bullet+}$ transfer occurs in <30 ps, the time scale of the overall reaction. Further restriction of the range of possible rates is complicated by the modest heterogeneity of the overall $FADH^{\bullet*}$ decay kinetics in WT PL, (but not in the mutant PL). Generally consistent with magic angle single wavelength measurements by Wang et al.[18] we found that a moderately better global fit was obtained with two close-lying components (10−20 ps and 30−50 ps) than with one (not shown). In general agreement with the assignment of the dispersion of the kinetics to protein heterogeneity,[18] we observed that the dispersion varies with proteins from different species (*E. coli* versus *Anacystis nidulans*). However, the results from our experiments on W→F mutant proteins argue against the proposed role of phenylalanine F_{366} as an ET bypass of Trp in a fraction of the proteins.[18]

Our results demonstrate that the three Trp chain in PL acts *effectively* as a "wire" within the protein, allowing efficient long-range electron transport. The speed of the overall reaction allows efficient competition with wasteful side reactions en route, such as deprotonation of $W_{359}^{\bullet+}$.[12] Close-lying aromatic amino acids have been identified as ET chains, sometimes coupled to proton transfer, in a variety of protein systems including cryptochrome blue light sensors[19] and ribonucleotide reductase.[20] Our results, making use of the natural photoactivation of DNA photolyase, indicate that they can act as effective wires to transfer electrons across proteins within tens of picoseconds.

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Figure 3. Comparison of anisotropy spectra $r(\lambda) = (\Delta A_{||} - \Delta A_{\perp})/(\Delta A_{||} + 2\Delta A_{\perp})$ with model spectra[13] as a function of the angle between the lowest $FADH^\bullet$ transition and the $Trp^{\bullet+}$ transition.