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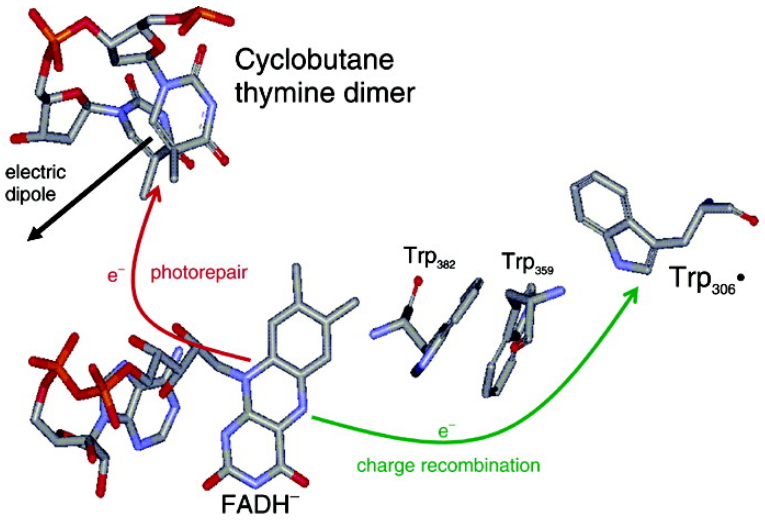

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Substrate Electric Dipole Moment Exerts a pH-Dependent Effect on Electron Transfer in *Escherichia coli* Photolyase

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We report evidence for the effect of the substrate electric field on charge recombination in the DNA repair enzyme *Escherichia coli* photolyase. Our results support the hypothesis that such an electric field can enhance the electron transfer (ET) from the enzyme to its substrate.[1]

E. coli photolyase is a blue-light photoreceptor and uses a light-driven electron-transfer mechanism to repair cyclobutane pyrimidine dimers (CPD) of DNA.[2] The active enzyme contains a fully reduced flavin adenine dinucleotide ($FADH^-$), which is located at the bottom of the substrate-binding cavity.[2,3] It forms a strong complex with CPD-containing DNA, which helps to stabilize $FADH^-$.[2,4,5] Its affinity for undamaged DNA is 5 orders in magnitude weaker.[2,4] The distance between $FADH^-$ and the CPD lesion has been estimated from van der Waals contact to ~15 Å.[1,3,6−9] In oxidized enzyme, MacFarlane and Stanley showed that the CPD binds sufficiently close to FAD for its electric dipole moment to induce an electrochromic shift of the FAD energy levels.[1] They proposed that this electric field could affect the ET between excited $^1FADH^-$ and CPD in the DNA repair process. We showed recently that in the unaltered enzyme, which contains a neutral radical semiquinone ($FADH^•$),[10,11] the substrate electric field also perturbs the energy levels and vibrational frequencies of $FADH^•$ in *E. coli* photolyase.[12] $FADH^•$ is an inactive form of the enzyme but can be activated to $FADH^-$ by photoreduction with Trp_{306} as final electron donor.[2,11,13−16,18] There is no evidence that this process plays a role in DNA repair in vivo.[2,18] The precise photoreduction mechanism is still a point of controversy. Several researchers favor super-exchange ET between excited $FADH^•$ and Trp_{306},[2,19] while others propose an electron-hopping model with Trp_{382} as the primary electron donor and Trp_{359} as an ET intermediate.[16,17] Mutation of Trp_{382} to phenylalanine to resolve this controversy has given conflicting results.[2,17,18] In the absence of external electron donors, charge recombination occurs on a millisecond time scale:[11,13,15,16] $FADH^- + Trp_{306}^• + H^+ \rightarrow FADH^• + TrpH_{306}$. Since $FADH^•$ in the unaltered enzyme is sensitive to the substrate electric field and undergoes photoinduced ET, it is an excellent system to test whether the substrate electric field can affect ET in *E. coli* DNA photolyase.

Figure 1 shows the charge recombination kinetics of $FADH^-$ and $Trp_{306}^•$ in *E. coli* DNA photolyase and its complex with UV-$p(dT)_{10}$ monitored at 580 nm at pH 7.4, 6.5, and 5.4.[20] The negative signal is due to ground-state bleaching of $FADH^•$, which recovers upon charge recombination. The charge recombination accelerates at lower pH, consistent with previous studies.[16] We measured monoexponential charge recombination kinetics, and the time constants are listed in Table 1. The same kinetics are observed at 510, 560, and 625 nm (data not shown). At pH 7.4, the charge recombination time increases 1.75-fold in the presence of substrate to $\tau^+ = 17.8 \pm 0.4$ ms, demonstrating that the substrate electric field does affect the ET reaction. At pH 6.5 it slows down by a factor of 1.3, and at pH 5.4 the effect is negligible (Table 1). The substrate electric field is still present at the lower pH values as indicated by the electrochromic shift of the S_0 to S_1 transition, which has a similar value as at pH 7.4 (Table 1).[12] The initial amplitude of the kinetic traces is virtually unchanged in the presence of substrate, indicating that the yield of the initial charge separation is relatively unaffected by its electric field. The presence of 12-fold excess undamaged $d(pT)_{10}$ does not affect the charge recombination kinetics in photolyase.

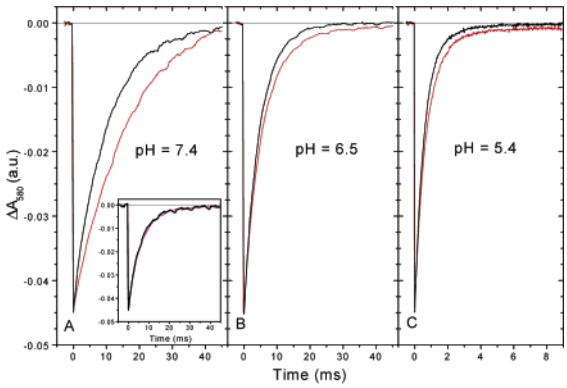

Figure 1. Transient absorption changes at 580 nm in 50 μM photolyase (black line) and its complex with UV-$p(dT)_{10}$ (red line) at pH 7.4 (A), 6.5 (B), and 5.4 (C). Inset: Photolyase in the absence (black line) and presence (red line) of undamaged $p(dT)_{10}$ at pH = 7.4.

Table 1. Substrate Electric Field Effect on Time Constants of Charge Recombination (τ) and the Electrochomic Shift in DNA Photolyase

pH	τ^- (ms)[a]	τ^+ (ms)[b]	τ^+/τ^-	ΔE (cm^{-1})[c]
7.4	10.2 ± 0.3	17.8 ± 0.4	1.75 ± 0.06	82 ± 8[d]
6.5	4.5 ± 0.2	5.7 ± 0.7	1.3 ± 0.2	79 ± 6
5.4	0.66 ± 0.03	0.75 ± 0.02	1.1 ± 0.1	89 ± 6

[a] Without substrate. [b] With substrate. [c] Electrochromic shift. [d] Reference 12.

We will analyze our results by using the semiclassical model of electron transfer with the ET rate, k_{et}, given by:[22]

$$k_{et} = \sqrt{\frac{4\pi^3}{h^2 \lambda k_B T}} H_{AB}^2 \exp\left[-\frac{(\Delta G^\circ + \lambda)^2}{4\lambda k_B T}\right] \qquad (1)$$

where h is Planck's constant, k_B is Boltzmann's constant, T is the temperature, λ is the reorganization energy, H_{AB} is the electronic coupling matrix element, and ΔG° the free energy change. Boxer and co-workers have extensively investigated the effect of applied electric fields on protein ET reactions.[23] The electric field can perturb both nuclear and electronic terms of eq 1,[23] suggesting that the substrate electric field can potentially affect H_{AB} and the Franck−Condon overlap between the initial and final states, which is accounted for in the exponential term. Since the substrate electric

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field of UV-p(dT)$_{10}$ at the FAD cofactor is relatively weak and the distance between FAD and Trp$_{306}$ relatively large (~13.5 Å), it is unlikely to modify H_{AB} significantly.[1,3,23] The change in the ET rate most likely arises from modification of the Franck−Condon overlap by the substrate electric field. An applied electric field modifies $\Delta G°$ by an energy ΔU given by: $\Delta U = \vec{E} \cdot \Delta\vec{\mu}_{et}$,[23] where $\vec{E}$ is the electric field, and $\Delta\vec{\mu}_{et}$ is the difference between the dipole of the products and the dipole of the reactants. At pH 5.4, both products (FADH$^{\bullet}$/TrpH) and reactants (FADH$_2$/Trp$^{\bullet}$) are charge neutral,[24] and ΔU is expected to be very small with no modification to $\Delta G°$ and k_{et}, as observed in our experiments. At pH 7.4, the products are still charge neutral, but one of the reactants (FADH$^-$/Trp$^{\bullet}$) is negatively charged, and the difference dipole moment $\Delta\vec{\mu}_{et}$ can be defined:[26] $\Delta\vec{\mu}_{et} = 2q\vec{r}_{et}$, where q is the charge transferred and $\vec{r}_{et}$ is the charge-transfer vector. For the charge recombination at pH 7.4, one can estimate ΔU to be −620 or −185 meV, depending on electric field strength.[1,27] Therefore, the substrate electric field can modify $\Delta G°$ of the charge recombination at pH 7.4 while at pH 5.4 its effect is negligible.

At pH 7.4, E_m(Trp$^{\bullet}$/TrpH) was measured to be 0.86 V,[28] while E_m(FADH$^-$/FADH$^{\bullet}$) in photolyase has been estimated between −0.33 and −0.5 V.[29] Therefore, $\Delta G°$ for the charge recombination reaction between FADH$^-$ and Trp$_{306}^{\bullet}$ in photolyase ranges from −1.19 to −1.36 eV. For protein ET, λ is between 0.74 and 1.0 eV,[30,31] and the charge recombination occurs in the Marcus inverted region.[22] The amount of change in $\Delta G°$ ($\Delta\Delta G°$) can be estimated by using the ratio of 1.75 for the ET rate without and with substrate, and $-120\ \text{meV} \le \Delta\Delta G° \le -35\ \text{meV}$.[32] These values are 1.5−18 times smaller than the estimated value of ΔU but do have the correct sign. Two factors may be responsible for this difference. First, the electric field is produced by an electric dipole, and its strength will diminish along the ET path, reducing ΔU. Second, the reorganization energy may be underestimated because of reprotonation of Trp$_{306}$. For free-energy optimized charge recombination ($-\Delta G° = \lambda$), $\Delta\Delta G° = -200$ meV for a 1.75-fold decrease in ET rate.[32] With λ being somewhat underestimated, $\Delta\Delta G°$ may be larger and have a value between −35 and −200 meV. Combined with an overestimation of ΔU, $\Delta\Delta G°$ is likely of the same order of magnitude as ΔU. An increase in ET distance of $\Delta R = 0.4$ Å could also explain the 1.75-fold change in ET rate but not the pH dependence, because substrate binding is unaffected by pH.

Our experimental data demonstrate for the first time that the CPD electric dipole moment affects ET in photolyase. ΔU calculated from electric field parameters and $\Delta\Delta G°$ estimated from the change in ET rate are in good agreement, indicating that the substrate electric dipole is responsible for the observed effect. However, stabilization of FADH$^-$ by the substrate electric field is significantly smaller in the presence of Trp$_{306}^{\bullet}$ than in its absence.[5] Although charge recombination is only affected by a factor of 1.75, our data support the proposal that the substrate electric field may enhance the physiologically important ET from FADH$^-$ to the CPD lesion,[1] providing a high photoreactivation yield following excitation. The electric field's physiological role may be to modify $\Delta G°$ for efficient productive ET from excited FADH$^-$ to CPD, so that unproductive ET to nonsubstrate molecules in the absence of a correct electric field that could render photolyase catalytically inactive will be less favorable, thereby protecting the enzyme against deactivation. Detailed knowledge of specific ET parameters is required to determine the effect of the CPD electric field on this physiologically important ET process[22,23] and are currently under investigation. Besides uracil DNA glycosylase, photolyase could be the second enzyme to utilize its substrate electrostatic field to facilitate DNA repair.[33]

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Supporting Information Available: Calculations of ΔU, $\Delta\Delta G°$, and ΔR and the electrochromic shift (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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