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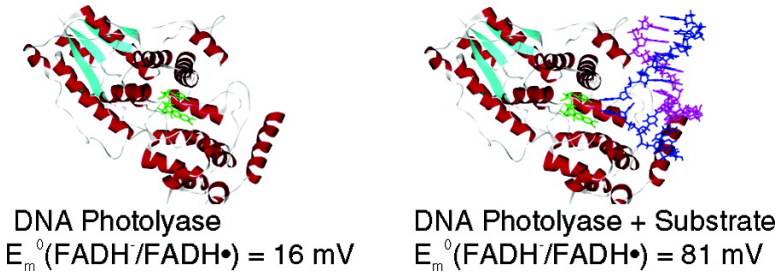

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Substrate Binding Modulates the Reduction Potential of DNA Photolyase

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The reduction potential of DNA photolyase in the absence of substrate is measured to be 16 mV vs NHE, which is significantly higher than estimates of −500 to −200 mV currently used in the literature.[1−5] This reduction potential increases by 65 mV upon substrate binding. This is the first measurement of a reduction potential for this class of DNA-repair enzymes.

DNA photolyase uses a light-driven electron transfer (ET) process to repair cyclobutane pyrimidine dimers (CPD), the most common type of photodamage, on DNA.[6] The FAD cofactor present in the enzyme has three possible oxidation states, FAD (fully oxidized), $FADH^{\bullet}$ (neutral semiquinone), and $FADH^{-}$ (fully reduced). The active enzyme requires $FADH^{-}$, but the enzyme is isolated as the $FADH^{\bullet}$ species.

Two ET processes occur in the enzyme: photoinduced DNA repair of substrate by the active form and photoreduction of the neutral semiquinone to the fully reduced active enzyme. In the catalytic cycle, $FADH^{-}$ donates an electron to the CPD substrate upon excitation, forming $FADH^{\bullet}$ and radical CPD anion. The CPD anion radical spontaneously forms monomer base and base anion radical with presumable electron transfer back to $FADH^{-}$. Controversy exists for the exact ET pathway and the full recovery of the $FADH^{-}$ species.[6−8] In the photoreduction process, the $FADH^{\bullet}$ state is photoreduced to $FADH^{-}$ with Trp_{306} as the terminal electron donor, but the biological significance of this process is unclear.[6,7] Controversy also exists to the exact ET pathway with both Trp_{382} and Phe_{366} cation radicals identified as potential ET intermediates.[1,6,9,10] In the absence of external electron donors, charge recombination occurs on a millisecond time scale,[1,3,11,12] but a recent study reported nanosecond charge recombination under aerobic conditions.[9] The CPD electric dipole moment also affects the ET process, presumably by modifying the electronic structure of the flavin cofactor.[3,13,14] The determination of $E_m(FADH^{-}/FADH^{\bullet})$ is a significant advancement and will allow for more realistic calculations and modeling of the ET processes and for tests of the viability of different pathways.

A wide range of reduction potentials are found in the literature for flavoproteins.[15] The reduction potential is strongly affected by the polarity of the binding pocket, electrostatic effects, $\pi-\pi$ interactions, FAD conformation, and hydrogen bonding.[16−20] The original estimated reduction potential[21] of −500 mV to −330 mV for DNA photolyase was based upon the observation that the $FADH^{-}$ state reacts with oxygen and that the lowest observed reduction potential for a flavoprotein is around −500 mV. Since that estimate, the reduction potential of the oxygen/superoxide couple has been revised upward from −0.33 to −0.137 V.[22]

We examined the reduction potential for the reaction between the neutral semiquinone and fully reduced forms of the enzyme: $FADH^{\bullet} + 1\ e^{-} \rightarrow FADH^{-}$, using a redox titration in the presence of mediators as described by Dutton.[23] The concentration of $FADH^{\bullet}$ was measured by monitoring absorption of $FADH^{\bullet}$ at 625 nm.[12] Nernst plots of typical data are shown in Figure 1 for enzyme solution alone along with enzyme solution containing a 10 times excess of UV-$p(dT)_{10}$, a UV-irradiated polythymidylate decamer. The least-squares fit is denoted by the line for each case. The midpoint potential of photolyase in the absence of substrate is 16 ± 6 mV vs NHE (average of eight data sets, error given is the standard deviation), and the enzyme in the presence of excess substrate has a midpoint potential of 81 ± 8 mV (average of five data sets, error given is the standard deviation). We also measured a midpoint potential of 28 ± 2 mV (average of two data sets) in the presence of a 10-fold excess of undamaged $p(dT)_{10}$. Both slopes shown in Figure 1 (65 ± 5 mV for enzyme alone, 62 ± 6 mV for enzyme with substrate) are consistent with one electron transfer. We did not see any indication of the fully oxidized state in our absorption spectra, and we have not attempted to measure the second reduction potential for the flavin; the enzyme stability significantly decreases upon further oxidation under the conditions used.

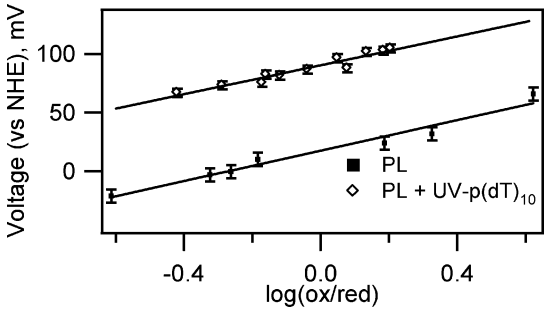

Figure 1. Nernst Plot of photolyase with and without UV-$p(dT)_{10}$. Each data set is a single experiment with the error bars calculated from least-squares analysis. Each sample contained 15 μM photolyase with 50 μM of each mediator, 0.29 M K_2SO_4, 35 mM Hepes, pH 7.0 at 10 °C. The R^2 values for the enzyme alone and enzyme with substrate are 0.98 and 0.96, respectively.

The standard reduction potential of $(FADH^{-}/FADH^{\bullet})$ is slightly higher than that of the $(O_2^{\bullet-}/O_2)$ couple, and $\Delta G° = 15$ kJ and $K = 1.9 \times 10^{-3}$ for the reaction between O_2 and $FADH^{-}$ at 10 °C. The oxidation will still readily occur due to the instability of the superoxide ion. Since the resulting $O_2^{\bullet-}$ will react readily with surrounding molecules and the O_2 concentration is constant, the $FADH^{-}$ will be driven toward $FADH^{\bullet}$ in the presence of oxygen. With substrate bound, $\Delta G° = 21$ kJ, and $K = 1.3 \times 10^{-4}$, and $FADH^{-}$ is more resistant to air oxidation.

The increase in midpoint potential upon substrate binding is not unexpected. Jordan and Jorns reported increased stability of fully reduced enzyme in the presence of oxygen when the substrate was present.[24] We have found several examples in the literature of substrate-induced redox changes in flavoproteins with changes that range from 10 to 160 mV.[25−31] For example, the 160 mV decrease in the $E_m(FADH^{-}/FAD)$ in D-amino acid oxidase has been attributed

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to a neutralization of a positive charge in the flavin binding pocket that occurs with binding of negatively charged substrate.[25] Acyl-CoA dehydrogenase also experiences a 100 mV increase in $E_m(FADH^-/FAD)$ upon substrate binding that appears to be due to a decrease in electron density near the isoalloxazine ring, a change in the polarity of the cofactor binding pocket.[32] Changes in $\pi-\pi$ interactions, FAD conformation, and/or hydrogen bonding that occur upon substrate binding are also expected to affect the reduction potential. In our specific case, the $FADH^-$ state appears to be stabilized relative to the semiquinone state by the substrate binding. The most likely candidate is an alteration of hydrogen bonding; we have published evidence that the hydrogen bonding to the cofactor changes upon substrate binding.[13]

In terms of the thermodynamics of the catalytic cycle, our measured value of $E_m(FADH^-/FADH^\bullet)$ decreases the energy gap between the CPD-bound $FADH^-$ singlet excited state and the $FADH^\bullet - CPD^{\bullet-}$ intermediate by ca. 0.4 eV compared to the value estimated earlier.[33] Our findings also prompted us to reevaluate our earlier calculations on the charge-recombination process following FADH• photoreduction: $FADH^- + Trp_{306}^\bullet + H^+ \rightarrow FADH^\bullet + Trp_{306}$.[3] By using $E_m(TrpH/Trp^\bullet) = 0.86$ V at pH 7.0[34] and a 1.75 ratio between the rate of charge recombination of photolyase in the absence and presence of UV-$p(dT)_{10}$,[3] we find $\Delta G^\circ = -0.844$ eV for photolyase and $\Delta G^\circ = -0.779$ eV for the enzyme−substrate complex. The reorganization energy, λ, for protein electron-transfer usually ranges from 0.7 to 1.0 eV,[35−37] and by assuming that substrate binding affects neither the electronic coupling between reactants and products nor their reorganization energies, we estimate that $\lambda = 1.44$ eV. This large value suggests that concerted electron−proton transfer may occur.[38] We ascribe the large reorganization energy mainly to the protonation of Trp_{306} during charge recombination with a smaller contribution from FAD of which the isoalloxazine ring transforms from a butterfly shape ($FADH^-$) to a more planar conformation (FADH•).[39] This analysis suggests that charge recombination occurs in the normal region.

The positive change in ΔG° upon substrate binding is opposite in sign to our earlier estimate of the effect of the substrate electric dipole on the charge recombination.[3] These calculations were based on a computer model of the enzyme−substrate complex.[40] The recent crystal structure of *Anacystis nidulans* in complex with repaired-DNA shows that a different computer model of the complex is more accurate.[41,42] Preliminary calculations on the basis of this new structural information yield a change in ΔG° of +210 meV due to the interaction with the substrate electric dipole (substrate electric field at FAD cofactor of about 1.0×10^8 V/m).[14] Although this value is larger than the one determined from the change in reduction potential, the fact that both have the same sign is an excellent qualitative result at this point.

In summary, we measure the midpoint potential of DNA photolyase to be much greater than estimated in the literature and that the reduction potential increases by 65 mV upon substrate binding. This modulation in reduction potential has only a minor effect on the ET processes.[35−37] The physiological relevance appears to be an increase in stability of the catalytic form of the enzyme with respect to oxidation.

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Supporting Information Available: Sample preparation, experimental details, and calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Aubert, C.; Vos, M. H.; Mathis, P.; Eker, A. P. M.; Brettel, K. *Nature* **2000**, *126*, 586−590.
(2) Popović, D. M.; Zmiriæ, A.; Zarić, S. D.; Knapp, E.-W. *J. Am. Chem. Soc.* **2002**, *124*, 3775−3782.
(3) Kapetanaki, S. M.; Ramsey, M.; Gindt, Y. M.; Schelvis, J. P. M. *J. Am. Chem. Soc.* **2004**, *126*, 6214−6215.
(4) Boussicault, F.; Krüger, O.; Robert, M.; Wille, U. *Org. Biomol. Chem.* **2004**, *2*, 2742−2750.
(5) Wang, H.; Saxena, C.; Quan, D.; Sancar, A.; Zhong, D. *J. Phys. Chem. B* **2005**, *109*, 1329−1333.
(6) Sancar, A. *Chem. Rev.* **2003**, *103*, 2203−2237 and references therein.
(7) Kavakli, I. H.; Sancar, A. *Biochemistry* **2004**, *43*, 15103−15110.
(8) MacFarlane, A. W., IV; Stanley, R. J. *Biochemistry* **2003**, *42*, 8558−8568.
(9) Saxena, C.; Sancar, A.; Zhong, D. *J. Phys. Chem. B* **2004**, *108*, 18026−18033.
(10) Byrdin, M.; Eker, A. P.; Vos, M. H.; Brettel, K. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 8676−8681.
(11) Heelis, P. F.; Payne, G.; Sancar, A. *Biochemistry* **1987**, *26*, 4634−4640.
(12) Gindt, Y. M.; Vollenbroek, E.; Westphal, K.; Sackett, H.; Sancar, A.; Babcock, G. T. *Biochemistry* **1999**, *38*, 3857−3866.
(13) Schelvis, J. P. M.; Ramsey, M.; Sokolova, O.; Tavares, C.; Cecala, C.; Connell, K.; Wagner, S.; Gindt, Y. M. *J. Phys. Chem.* **2003**, *107*, 12352−12362.
(14) MacFarlane, A., IV; Stanley, R. J. *Biochemistry* **2001**, *40*, 15203−15214.
(15) Stankovich, M. T. In *Chemistry and Biochemistry of Flavoenzymes*; Muller, F., Ed.; CRC Press: Boca Raton, FL, 1991; Vol. I., pp 401−425.
(16) Swenson, R. P.; Krey, G. D. *Biochemistry* **1994**, *33*, 8505−8514.
(17) Zhou, Z.; Swenson, R. P. *Biochemistry* **1995**, *34*, 3183−3192.
(18) Zhou, Z.; Swenson, R. P. *Biochemistry* **1996**, *35*, 15980−15988.
(19) Faro, M.; Gómez-Moreno, C.; Stankovich, M.; Medina, M. *Eur. J. Biochem.* **2002**, *269*, 2656−2661.
(20) Nogués, I.; Campos, L. A.; Sancho, J.; Gómez-Moreno, C.; Mayhew, S. G.; Medina, M. *Biochemistry* **2004**, *43*, 15111−15121.
(21) Heelis, P. F.; Deeble, D. J.; Kim, S.-T.; Sancar, A. *Int. J. Radiat. Biol.* **1992**, *62*, 137−143.
(22) Petlicki, J.; van de Ven, T. G. M. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 2763−2767.
(23) Dutton, P. L. *Methods Enzymol.* **1978**, *54*, 411−435.
(24) Jordan, S. P.; Jorns, M. S. *Biochemistry* **1988**, *27*, 8915−8923.
(25) Van den Berghe-Snorek, S.; Stankovich, M. T. *J. Biol. Chem.* **1985**, *206*, 3373−3379.
(26) Einarsdottir, G. H.; Stankovich, M. T.; Tu, S.-C. *Biochemistry* **1988**, *27*, 3277−3285.
(27) Stankovich, M. T.; Soltysik, S. *Biochemistry* **1987**, *26*, 2627−2632.
(28) Donnelly Lenn, N.; Stankovich, M. T.; Liu, H.-W. *Biochemistry* **1990**, *29*, 3709−3715.
(29) Becker, D. F.; Thomas, E. A. *Biochemistry* **2001**, *40*, 4714−4721.
(30) Fullerton, S. W. B.; Daff, S.; Sanders, D. A. R.; Ingledew, W. J.; Whitfield, C.; Chapman, S. K.; Naismith, J. H. *Biochemistry* **2003**, *42*, 2104−2109.
(31) Negri, A.; Tedeschi, G.; Ceciliani, F.; Ronchi, S. *Biochim. Biophys. Acta* **1999**, *1431*, 212−222.
(32) Pellett, J. D.; Sabaj, K. M.; Stephens, A. W.; Bell, A. F.; Wu, J.; Tonge, P. J.; Stankovich, M. T. *Biochemistry* **2000**, *39*, 13982−13992.
(33) Heelis, P. F.; Hartman, R. F.; Rose, S. D. *Chem. Soc. Rev.* **1995**, *24*, 289−297.
(34) Tommos, C.; Skalicky, J. J.; Piloud, D. L.; Wand, A. J.; Dutton, P. L. *Biochemistry* **1999**, *38*, 9495−9507.
(35) Page, C. C.; Moser, C. C.; Chen, X.; Dutton, P. L. *Nature* **1999**, *402*, 47−52.
(36) Gray, H. B.; Winkler, J. R. In *Electron Transfer in Chemistry*; Balzani, V., Ed.; Wiley-VCH: Weinheim, 2001; pp 3−23.
(37) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265−322.
(38) Sjödin, M.; Styring, S.; Wolpher, H.; Xu, Y.; Sun, L.; Hammarström, L. *J. Am. Chem. Soc.* **2005**, *127*, 3855−3863.
(39) Muller, F. In *Chemistry and Biochemistry of Flavoenzymes*; Muller, F., Ed.; CRC Press: Boca Raton, FL, 1991; Vol. I, pp 1−71.
(40) Sanders, D. B.; Wiest, O. *J. Am. Chem. Soc.* **1999**, *121*, 5127−5134.
(41) Mees, A.; Klar, T.; Gnau, P.; Hennecke, U.; Eker, A. P. M.; Carell, T.; Essen, L.-O. *Science* **2004**, *306*, 1789−1793.
(42) Antony, J.; Medvedev, D. M.; Stuchebrukhov, A. A. *J. Am. Chem. Soc.* **2000**, *122*, 1057−1065.

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