Vectorial Photoinduced Electron-Transfer and Charge Separation in a Zn(II)-Protoporphyrin−Bipyridinium Dyad Reconstituted Myoglobin

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The understanding of long-range electron transfer in proteins has been the subject of extensive theoretical[1] and experimental[2,3] research efforts. The donor−acceptor distances[4,5] within the proteins environmental[6] and structural parameters[7] were found to control the electron-transfer rates in the protein systems. The unique organization of the donor−acceptor units in the photosynthetic reaction center leads to vectorial electron transfer and effective charge separation.[8] Previous research efforts were directed to the organization of molecular[9,10] or supramolecular[11] donor−acceptor dyad, triad, and so forth assemblies which mimic the photosynthetic reaction center. The steric flexibility of many of the molecular dyads or triads prevents charge separation due to the rapid recombination of the spatially intimate redox products. Immobilization of molecular dyads (or triads) in protein systems could structurally rigidify the donor−acceptor pairs and lead to charge separation. Reconstitution of apo-hemoproteins, e.g., apomyoglobin, with the photoactive Zn(II)-protoporphyrin IX and site-specific covalent linkage of acceptor sites to the proteins, yield structurally defined donor−acceptor systems.[5] Also, reconstitution of apo-hemo proteins,[12] e.g., apo-myoglobin or apohemoglobin, with Co(II)-protoporphyrin IX, acting as an electron acceptor/catalytic center and the covalent attachment of a chromophore to the protein was reported to yield organized assemblies for controlled electron transfer. An alternative approach to construct donor−acceptor systems was addressed by Hamachi and Shinkai[13,14] and includes the reconstitution of apo-myoglobin with a chromophore-modified Fe(III)-protoporphyrin IX. This approach was further developed by the reconstitution of apo-myoglobin with a chromophore−quinone dyad[15a] or with a functionalized Zn(II)-protoporphyrin IX capable of generating a noncovalent, supramolecular complex with an electron acceptor.[15b] Here we wish to report on the reconstitution of apo-myoglobin with a Zn-(II)-protoporphyrin IX−bis-*N*,*N*′-dialkyl-4,4′-bipyridinium, Zn-(II)-P−V^{2+}, donor−acceptor dyad. We reveal that the reconstituted protein in the presence of $Ru(NH_3)_6^{3+}$, as secondary electron acceptor, mimics functions of the photosynthetic reaction center.

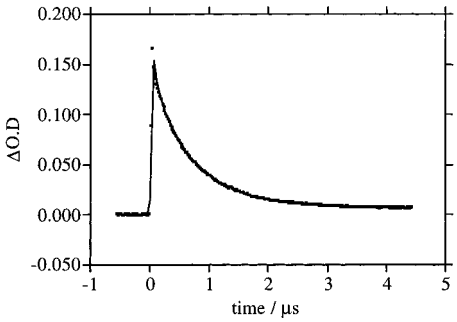
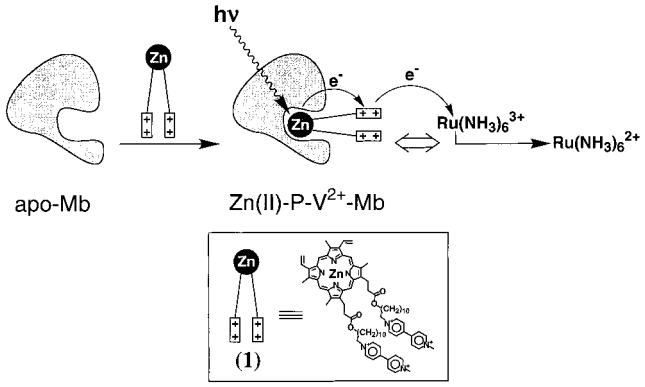


Figure 1. Transient formation and decay of the triplet state of Zn(II)-P−V^{2+}−Mb, 7×10^{-5} M, in 0.01 M phosphate buffer, pH = 7.3, followed at $\lambda = 470$ nm. Excitation wavelength $\lambda_{ex} = 550$ nm.

Scheme 1. Reconstitution of Apo-myoglobin with a Zn(II)-protoporphyrin IX Dyad and Vectorial Photoinduced Electron Transfer in the Assembly



Apo-myoglobin was reconstituted with Zn(II)-P−V^{2+}, (**1**), Scheme 1. Photoexcitation of the donor−acceptor reconstituted protein yields the triplet excited state. Figure 1 shows the triplet decay as a function of time. By comparison of the triplet decay rate in Zn(II)-P−V^{2+}−Mb to the triplet decay of Zn(II)-protoporphyrin reconstituted myoglobin, Zn(II)-P-Mb, lacking the acceptor units ($\tau = 110$ s^{-1}) we calculated the intramolecular electron-transfer quenching rate constant, eq 1, to be $k_q = 1.55 \times 10^6$ s^{-1}. The quenching of the triplet-state leads to intra-

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(1) (a) Marcus, R. A. *J. Chem. Phys.* **1956**, *24*, 966. (b) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265. (c) Bixon, M.; Jortner, J.; Michel-Beyerle, M. E.; Ogrodnik, A. *Biochim. Biophys. Acta* **1989**, *977*, 273.

(2) (a) McLendon, G. *Acc. Chem.* **1988**, *21*, 160. (b) McLendon, G.; Guarr, T.; McGuire, M.; Simolo, K.; Strauch, S.; Taylor, K. *Coord. Chem. Rev.* **1985**, *64*, 113.

(3) (a) Winkler, J. R.; Gray, H. B. *Chem. Rev.* **1992**, *92*, 369. (b) Isied, S. S.; Ogawa, M. Y.; Wishart, J. W. *Chem. Rev.* **1992**, *92*, 381.

(4) (a) Gray, H. B.; Malmström, B. G. *Biochemistry* **1989**, *19*, 7499. (b) Beratan, D. N.; Betts, J. N.; Onuchic, J. N. *Science* **1991**, *252*, 1285. (c) Skourtis, S. S.; Regan, J. J.; Onuchic, J. N. *J. Phys. Chem.* **1994**, *98*, 3379.

(5) (a) Axup, A. W.; Albin, M.; Mayo, S. L.; Crutchley, R. J.; Gray, H. B. *J. Am. Chem. Soc.* **1988**, *110*, 435. (b) Casimiro, D. R.; Wong, L.-L.; Colón, J. L.; Zewert, T. E.; Richards, J. H.; Chang, I.-J.; Winkler, J. R.; Gray, H. B. *J. Am. Chem. Soc.* **1993**, *115*, 1485.

(6) (a) Liang, N.; Grant Mauk, A.; Pielak, G. J.; Johnson, J. A.; Smith, M.; Hoffman, B. M. *Science* **1988**, *240*, 311. (b) McLendon, G.; Miller, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 7811.

(7) Conrad, D. W.; Zhang, H.; Stewart, D. E.; Scott, R. A. *J. Am. Chem. Soc.* **1992**, *114*, 9909.

(8) (a) Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. *J. Mol. Biol.* **1984**, *180*, 385. (b) Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. *Nature* **1985**, *318*, 618. (c) Chang, C.-H.; Tiede, D.; Tang, J.; Smith, U.; Norris, J.; Schiffer, M. *FEBS Lett.* **1986**, *205*, 82.

(9) (a) Connolly, J. S.; Bolton, J. R. In *Photoinduced Electron Transfer, Part D*; Fox, M. A., Chanon, M., Eds.; Elsevier: Amsterdam, 1988; Section 6.2. (b) Gust, D.; Moore, T. A. *Top. Curr. Chem.* **1991**, *159*, 103. (c) Kurreck, H.; Huber, M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 849. (d) Meyer, T. J. *Acc. Chem. Res.* **1989**, *22*, 163.

(10) (a) Gust, D.; Moore, T. A.; Moore, A. L.; Seely, G.; Liddell, P.; Barett, D.; Harding, L. O.; Ma, X. C.; Lee, S. J.; Gao, F. *Tetrahedron* **1989**, *45*, 4867. (b) Wasielewski, M. R. *Chem. Rev.* **1992**, *92*, 435.

(11) (a) David, E.; Born, R.; Kaganer, E.; Joselevich, E.; Dürr, H.; Willner, I. *J. Am. Chem. Soc.* **1997**, *119*, 7778. (b) Gubelmann, M.; Lehn, J.-M.; Sessler, J. L.; Harriman, A. *J. Phys. Chem.* **1990**, *94*, 308. (c) Harriman, A.; Kubo, Y.; Sessler, J. L. *J. Am. Chem. Soc.* **1992**, *114*, 388. (d) Sessler, J. L.; Wang, B.; Harriman, A. *J. Am. Chem. Soc.* **1993**, *115*, 10418.

(12) (a) Zahavy, E.; Willner, I. *J. Am. Chem. Soc.* **1996**, *118*, 12499. (b) Zahavy, E.; Willner, I. *J. Am. Chem. Soc.* **1995**, *117*, 10581.

(13) Hamachi, I.; Matsugi, T.; Tanaka, S.; Shinkai, S. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1657.

(14) (a) Hamachi, I.; Tanaka, S.; Shinkai, S. *Chem. Lett.* **1933**, 1417. (b) Hamachi, I.; Tanaka, S.; Shinkai, S. *J. Am. Chem. Soc.* **1993**, *115*, 10458.

(15) (a) Hayashi, T.; Takimura, T.; Ohara, T.; Hitomi, Y.; Ogoshi, H. *J. Chem. Soc., Chem. Commun.* **1995**, 2503. (b) Hayashi, T.; Takimura, T.; Ogoshi, H. *J. Am. Chem. Soc.* **1995**, *117*, 11606.

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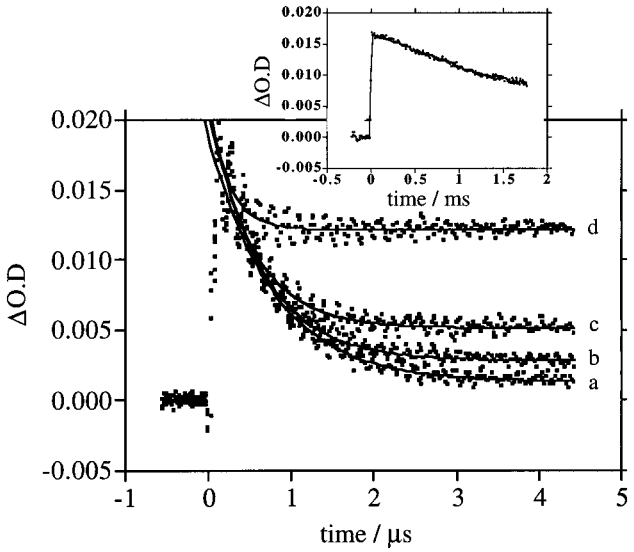

Figure 2. Transient recombination of the photogenerated redox species Zn-P$^{+\bullet}$−V$^{+\bullet}$ (a) in the absence of Ru(NH$_3$)$_6^{3+}$, and in the presence of Ru(NH$_3$)$_6^{3+}$, (b) 0.001 M, (c) 0.01 M, (d) 0.03 M. Inset: Transient decay of the diffusional recombination between Zn-P$^{+\bullet}$−V^{2+} and Ru(NH$_3$)$_6^{2+}$. Transients recorded in 0.01 M phosphate buffer, pH = 7.3, λ_{ex} = 550 nm, and at λ = 670 nm.

molecular electron transfer, and the photogenerated redox intermediates recombine, eq 2.

$$^{T}\text{Zn(II)-P-V}^{2+}\text{-Mb} \xrightarrow{k_q} \text{Zn(II)-P}^{+\bullet}\text{−V}^{+\bullet}\text{−Mb} \qquad (1)$$

$$\text{Zn(II)-P}^{+\bullet}\text{−V}^{+\bullet}\text{−Mb} \xrightarrow{k_b} \text{Zn(II)-P−V}^{2+}\text{−Mb} \qquad (2)$$

Figure 2, curve a, shows the transient recombination of the photogenerated redox species. The recombination process follows first-order kinetics, k_b= 1.3 × 10^6 s^{-1}. Photoexcitation of the photosensitizer−bipyridinium dyad, **1**, in the absence of the proteins, does not yield any triplet excited state and does not lead to observable redox-species. In addition, the parent Zn(II)-protoporphyrin IX exhibits fluorescence, λ_{em} = 680 nm, its fluorescence is completely quenched in the photosensitizer−electron acceptor dyad system. These photophysical properties of **1** are attributed to the effective quenching of the excited singlet state by the bipyridinium acceptor units. Preliminary molecular mechanics calculations indicate that the minimum energy structure of **1** consists of the sandwiched-type assembly where the Zn(II)-protoporphyrin unit is interlocked between the two bipyridinium units. The proximity of the components leads to the effective quenching of the excited singlet state and to the lack of observable redox products. Reconstitution of the Zn(II)-P−V^{2+} dyad, **1**, into apo-myoglobin, spatially separates the photosensitizer from the acceptor sites. This leads to the singlet−triplet crossing, quenching of the long-lived triplet state and the effective charge separation of the redox photoproducts exhibiting a lifetime of τ = 770 ± 30 ns. Using the k_q and k_b values, and the respective redox-potentials (vs NHE) of the excited triplet state, (E° (Zn−P$^{+\bullet}$/Zn−P^{T}) = −1.98 V) the bipyridinium units (E° (V^{2+}/V$^{+\bullet}$) = −0.43 V) and the Zn(II)-protoporphyrin component (E° (Zn−P$^{+\bullet}$/Zn−P) = 0.66 V), we estimate the reorganization energy accompanying electron transfer to be λ = 1.3 ± 0.1 eV

Force-field calculations indicate that the dyad **1** reconstitutes in apo-myoglobin through a channel and that upon reconstitution, the two bipyridinium electron-acceptor units block the channel gate at the protein periphery. This feature introduces an electrostatic barrier that can further stimulate vectorial electron transfer and induce charge separation, Scheme 1. As secondary electron acceptor we utilize Ru(NH$_3$)$_6^{3+}$ (E° = 0.083 V vs NHE). Figure 2 shows the transient decay curves of the oxidized photoproduct in the absence of Ru(NH$_3$)$_6^{3+}$ (curve a) and at different concentrations of the secondary acceptor (curves b−d). As the concentration of Ru(NH$_3$)$_6^{3+}$ increases, the fraction of the Zn−P$^{+\bullet}$ decaying by the fast process decreases, and a population of oxidized species not decaying at this time scale increases. At a Ru(NH$_3$)$_6^{3+}$ concentration corresponding to 3 × 10^{-2} M, the fast decaying component is completely depleted, and the oxidized species does not decay at this time domain. The oxidized species decays, however, at a substantially longer time scale in the millisecond range, Figure 2 (inset). These results are explained by the operation of a vectorial electron transfer that electrostatically controls the recombination process and facilitates charge separation, Scheme 1.

The reduced photoproduct of the generated redox species mediates the reduction of Ru(NH$_3$)$_6^{3+}$, eq 3. This reaction

$$\begin{array}{l} \text{Zn(II)-P}^{+\bullet}\text{-V}^{+\bullet}\text{-Mb} + \text{Ru(NH}_3\text{)}_6^{3+} \xrightarrow{k_2} \text{Zn(II)-P}^{+\bullet}\text{-V}^{2+}\text{-Mb} + \text{Ru(NH}_3\text{)}_6^{2+} \\ \quad \downarrow k_b \\ \quad \text{Zn(II)-P-V}^{2+}\text{-Mb} \end{array} \qquad (3)$$

competes with the intraprotein back-electron transfer, and as the concentration of Ru(NH$_3$)$_6^{3+}$ is elevated, the recombination decreases. At a Ru(NH$_3$)$_6^{3+}$ concentration of 3 × 10^{-2} M only the mediated directional electron-transfer cascade is operative. Knowing the fraction of oxidized species that recombines by the intraprotein pathway, we estimate the mediated electron-transfer rate constant to be k_2 = (6.5 ± 0.5) × 10^7 M^{-1}•s^{-1}.

The secondary reduced acceptor, Ru(NH$_3$)$_6^{2+}$ is electrostatically repelled by the bipyridine barrier gate. As a result the diffusional recombination of the redox species, eq 4,

$$\text{Zn(II)-P}^{+\bullet}\text{−V}^{2+}\text{−Mb} + \text{Ru(NH}_3\text{)}_6^{2+} \xrightarrow{k_b} \text{Zn(II)-P−V}^{2+}\text{−Mb} + \text{Ru(NH}_3\text{)}_6^{3+} \qquad (4)$$

is very slow, and the generated redox species reveal unprecedented high stability (ca. 2.6 ms).[16]

In conclusion, we have demonstrated novel means to design a model system for the photosynthetic reaction center. Reconstitution of a photosensitizer−acceptor dyad in apo-myoglobin, spatially separates the donor−acceptor components and the resulting redox species. This already stabilizes the intermediate redox photoproducts that reveal a lifetime of 770 ± 30 ns. Coupling of these redox intermediates to a secondary electron acceptor stimulates a vectorial electron-transfer cascade. Electrostatic repulsion of the secondary reduced product from the protein and steric blocking of the recombination path with the oxidized species yields photoredox species of unprecedented stability.

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Supporting Information Available: ^{1}H-NMR spectrum of **1** and absorption spectrum of Zn(II)-P−V^{2+}−Mb (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) Control experiments reveal that the triplet lifetime of ^{T}Zn(II)-P−V^{2+}−Mb is not affected by the addition of Ru(NH$_3$)$_6^{3+}$, 0.03 M, indicating that the intermolecular quenching, eq 5, is not operative. This is probably due to the electrostatic repulsion of Ru(NH$_3$)$_6^{3+}$ by the bipyridinium units.

$$^{T}\text{Zn(II)-P-V}^{2+}\text{-Mb} + \text{Ru(NH}_3\text{)}_6^{3+} \not\longrightarrow \text{Zn(II)-P}^{+\bullet}\text{-V}^{2+}\text{-Mb} + \text{Ru(NH}_3\text{)}_6^{2+} \qquad (5)$$