Eosin-Modified Reconstituted Co(II) Protoporphyrin IX Myoglobin: A Semisynthetic Photoenzyme for H_2 Evolution and Hydrogenation

Itamar Willner,* Eran Zahavy, and Vered Heleg-Shabtai

Institute of Chemistry and
Farkas Center for Light-Induced Processes
The Hebrew University of Jerusalem
Jerusalem 91904, Israel

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Photoactivation of proteins[1,2] and specifically of enzymes[3,4] has recently been a subject of intensive research efforts. Light-induced electron transfer in photosensitizer-modified redox proteins provides a general method to probe intra-protein electron transfer rates. Recently, we demonstrated that chemical modification of a redox enzyme such as glutathione reductase by the eosin chromophore turns the native enzyme into a photoactive biocatalyst.[5] That is, the enzyme is activated by light rather than by the native cofactor toward its specific biotransformation. Here we wish to report on a novel approach to designing photoenzymes that includes the semisynthetic modification of the myoglobin apoprotein with catalytic and photoactive sites.[6]

The eosin-modified Co(II)-reconstituted myoglobin, Eo^{2-}-Mb-Co(II), was prepared by the sequence of reactions outlined in Scheme 1. The Fe(II) heme center was extracted from native myoglobin, and the Co(II) protoporphyrin IX was implanted into the apoprotein.[7] The resulting Co(II) protoporphyrin IX-reconstituted myoglobin was reacted with eosin isothiocyanate to yield Eo^{2-}-Mb-Co(II). From the absorbance of the resulting protein (λ = 526 nm, ϵ = 83 000 $M^{-1}cm^{-1}$), the loading of Eo^{2-} corresponds to 2 units/protein unit. Time-resolved laser flash photolysis experiments reveal that the eosin triplet is quenched by the Co(II) protoporphyrin IX site in the Eo^{2-}-Mb-Co(II) assembly by an electron transfer pathway, eq 1. The resulting photoproducts ($Eo^{\bullet -}$, λ = 460 nm, and Co(I) protoporphyrin IX, λ = 404 nm) decay by the thermodynamically favored back-reaction, eq 2. Addition of Na_2EDTA to the system eliminates the back-reaction by competitive oxidation of the sacrificial electron donor, and the Co(I) protoporphyrin IX is accumulated in the system, eq 3.[8]

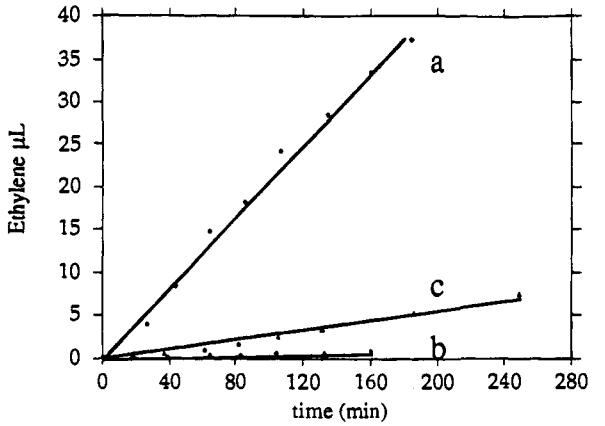

Figure 1. Rate of acetylene hydrogenation to ethylene at time intervals of illumination: (a) Eo^{2-}-Mb-Co(II) system, 0.5 mg·mL^{-1} of protein; (b) Mb-Co(II) and Eo^{2-} system, 0.5 mg·mL^{-1} of protein; (c) Co(II) protoporphyrin IX and Eo^{2-} system. In all systems were used the bulk concentrations of Co(II), 2.5 × 10^{-5} M and Eo^{2-}, 5 × 10^{-5} M. All systems consist of 3 mL of phosphate buffer, pH = 7.5, that contains Na_2EDTA, 1 × 10^{-2} M.

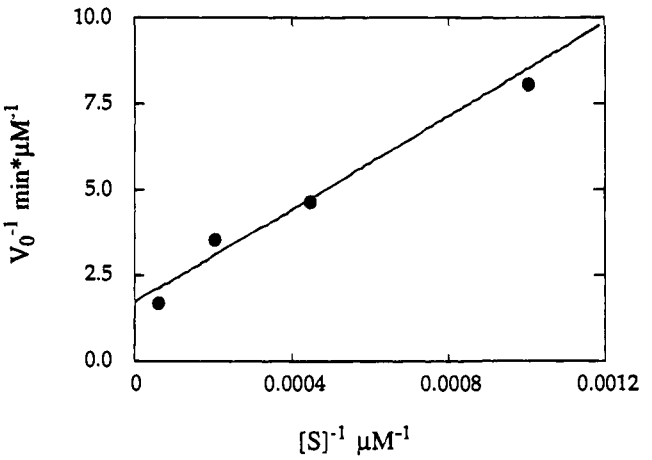

Figure 2. Lineweaver−Burk plot for the photohydrogenation of acetylene dicarboxylic acid in a photosystem consisting of Eo^{2-}-Mb-Co(II) and Na_2EDTA, 1 × 10^{-2} M.

$$^{T}Eo^{2-}\text{-Mb-Co(II)} \xrightarrow{k_q = 5.2 \times 10^4\ s^{-1}} Eo^{\bullet -}\text{-Mb-Co(I)} \quad (1)$$

$$Eo^{\bullet -}\text{-Mb-Co(I)} \xrightarrow{k_b = 1.4 \times 10^5\ s^{-1}} Eo^{2-}\text{-Mb-Co(II)} \quad (2)$$

$$Eo^{\bullet -}\text{-Mb-Co(I)} + Na_2EDTA \longrightarrow Eo^{2-}\text{-Mb-Co(I)} + Na_2EDTA_{ox} \longrightarrow \text{decomposition product} \quad (3)$$

Illumination of Eo^{2-}-Mb-Co(II) in an aqueous solution, pH = 7.5, that includes Na_2EDTA, 1 × 10^{-2} M, results in H_2 evolution (ϕ = 2 × 10^{-4}). Illumination of this system under acetylene, C_2H_2, eliminates the formation of hydrogen, and C_2H_2 is hydrogenated to ethylene. Figure 1 (curve a) shows the rate of C_2H_4 evolution at time intervals of illumination, λ > 475 nm. Control experiments revealed that H_2 evolution and photohydrogenation of C_2H_2 are eliminated upon exclusion of Na_2EDTA from the systems. The quantum yield for C_2H_4 formation corresponds to ϕ = 10^{-2}. For comparison, the rates of C_2H_4 evolution from two control photosystems consisting of Mb-Co(II) and diffusional eosin (curve b) and Co(II) protoporphyrin IX (lacking the protein) and diffusional eosin (curve c) are also shown. The rate of C_2H_4 formation in the

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(6) For recent examples of electron transfer in reconstituted semisynthetic myoglobin, cf.: (a) Hamachi, I.; Tanaka, S.; Shinkai, S. *J. Am. Chem. Soc.* **1993**, *115*, 10458. (b) Hamachi, I.; Tajiri, Y.; Shinkai, S. *J. Am. Chem. Soc.* **1994**, *116*, 7437.

(7) (a) Yonetani, T.; Yamamoto, H.; Woodrow, G. V. *J. Biol. Chem.* **1974**, *249*, 682. (b) Hamachi, I.; Nakamura, K.; Fujita, A.; Kunitake, T. *J. Am. Chem. Soc.* **1993**, *115*, 4966.

(8) The time-resolved laser experiments reveal that the second $^{T}Eo^{2-}$ unit, associated with the protein, is self-quenched by ground-state Eo^{2-} formed by the back electron transfer. The detailed analysis of these results will be provided in a later report.

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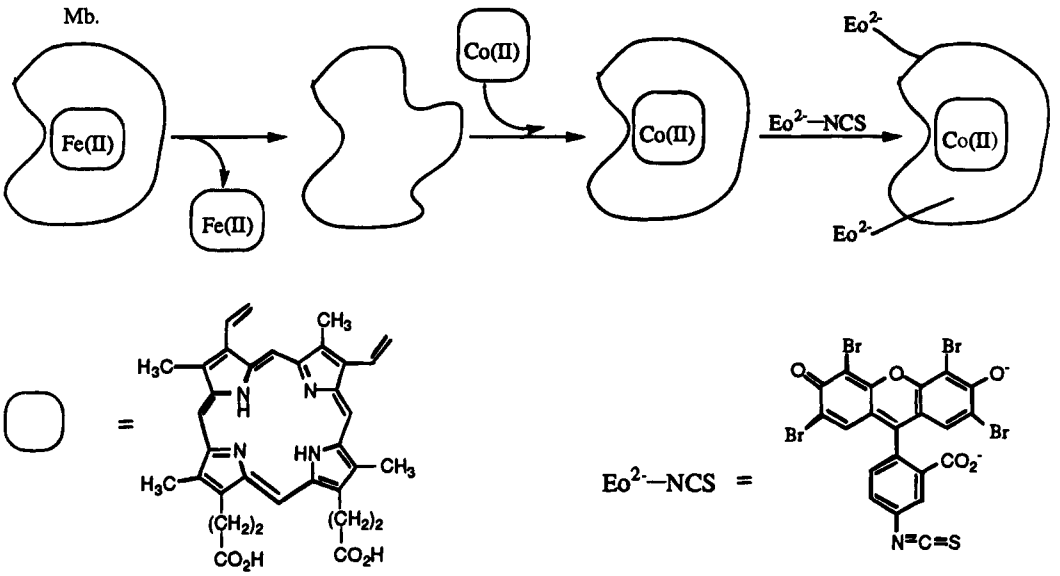
Scheme 1. Reconstitution of Eo^{2-}-Mb-Co(II)



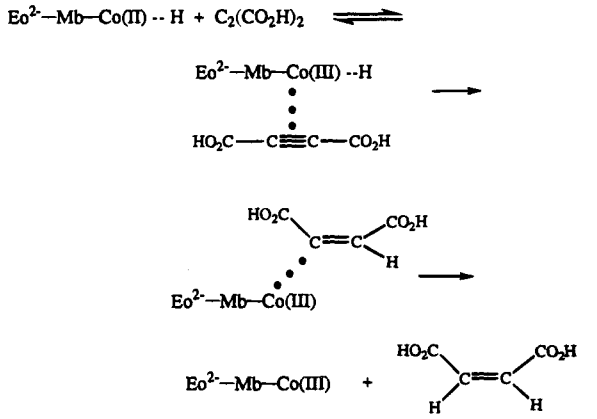
Eo^{2-}-Mb-Co(II) system is 70-fold and 10-fold higher than that from Mb-Co(II) lacking the covalently linked Eo^{2-} system and the system consisting of diffusional Eo^{2-} and Co(II) protoporphyrin IX that lacks the protein, respectively. Time-resolved flash photolysis experiments revealed that inefficient electron-transfer quenching[9] of $^{T}Eo^{2-}$ by Mb-Co(II) and fast diffusion-controlled recombination of the redox photoproducts in the protein-lacking system[10] lead to the inefficient performance of the reference systems. Thus, organization of the photosensitizer and electron acceptor within the protein provides an assembly for effective electron-transfer quenching and for the stabilization of the photoproducts against back electron-transfer. This effective charge separation enhances the subsequent catalyzed transformation as compared to the reference systems. The H_2 evolution and hydrogenation of acetylene are attributed to the formation of cobalt hydride species, eqs 4 and 5. H_2 evolution

$$Eo^{2-}\text{-Mb-Co(I)} \xrightarrow{+H^+} Eo^{2-}\text{-Mb-Co(III)-H} \xrightarrow{H^+} Eo^{2-}\text{-Mb-Co(III)} + H_2 \quad (4)$$

$$Eo^{2-}\text{-Mb-Co(III)-H} + C_2H_2 \rightarrow Eo^{2-}\text{-Mb-Co(III)-}C_2H_3 \xrightarrow{H^+} Eo^{2-}\text{-Mb-Co(III)} + C_2H_4 \quad (5)$$

originates from the protonation of the Co—H intermediate.[11] Insertion of C_2H_2 into Co—H species kinetically competes with hydrogen evolution and leads to its hydrogenation to C_2H_4. The effective hydrogenation of acetylene as compared to the protonation of the Co—H and H_2 evolution is reflected in the substantially higher quantum yield for ethylene formation. It should be noted that in a further control experiment that included Co(II) protoporphyrin IX, Eo^{2-}, and albumin as mute protein additive, the yield of photoinduced ethylene formation was similar to that observed in the protein-lacking system. This result implies that the protein additive does not affect the photoinduced hydrogenation by itself but rather the specific position of Co(II) protoporphyrin IX in the heme site of Mb controls the photocatalyzed transformation.

Photoinduced hydrogenation of acetylene dicarboxylic acid (ADA) by Eo^{2-}-Mb-Co(II) in the presence of Na_2EDTA revealed two important results: photohydrogenation of ADA produced selectively maleic acid (>92%) and only trace amounts of fumaric acid were formed (<8%). Analysis of the photohydrogenation rates at different ADA concentrations revealed that the Eo^{2-}-Mb-Co(II) system follows the Michaelis—Menten kinetic model, Figure 2, $K_m = 4$ mM, $V_{max} = 0.6$ μM/min. Thus, the Co(II)-protoporphyrin IX center associated with Mb presumably generates an active site where orientation of the Co—H insertion product is induced by the protein to stimulate the selective formation of maleic acid, eq 6.



We thus conclude that reconstitution of proteins by a chromophore and a redox-active center provides a general means to tailor photoenzymes. The activity of these assemblies takes advantage of the protein matrix in controlling charge separation and stimulating of selective chemical transformations. Preliminary studies reveal that Eo^{2-}-Mb-Co(II) acts as photocatalyst for CO_2 fixation to formate and vitamin B_{12} mimicking transformations.

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(9) The quenching rate constant of $^{T}Eo^{2-}$ by Mb-Co(II) corresponds to $K_q = 8 \times 10^6$ $M^{-1}s^{-1}$.

(10) The recombination rate constant of Co(I) protoporphyrin IX with $Eo^{\bullet-}$ corresponds to $k_b = 4 \times 10^8$ $M^{-1}s^{-1}$.

(11) For other H_2 evolution systems by intermediary Co—H species, cf.: (a) Brown, G. M.; Brunschwig, B. S.; Creutz, C.; Endicott, J. F.; Sutin, N. *J. Am. Chem. Soc.* **1979**, *101*, 1298. (b) Krishnan, C. V.; Creutz, C.; Mahajan, D.; Schwarz, H. A.; Sutin, N. *Isr. J. Chem.* **1982**, *22*, 98.