Application of Eosin-Modified Reconstituted Co(II)-Myoglobin as Semisynthetic Photoenzyme for Cyclic Photosynthesis

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During the last two decades, extensive research efforts were directed to the development of artificial photosynthetic systems.[1,2] Significant progress was made in the design of molecular assemblies[3] and microheterogeneous[4,5] systems where the light-generated redox intermediates are stabilized against back electron transfer. Also, various heterogeneous and homogeneous catalysts were applied to promote photosynthetic reactions by the photogenerated redox intermediates.[6,7] In these systems, reductive or oxidative transformations were explored using sacrificial electron donors, or electron acceptors.[1] The mandatory need for a sacrificial component originates from the back-electron-transfer processes of the reductive and oxidative sites. Enzymes were also applied as biocatalysts for photosynthetic transformations,[8,9] and recently, we demonstrated that chemical modification of redox enzymes by a photosensitizer leads to electrical communication between the excited chromophore and the active site and yields a novel class of biocatalysts−photoenzymes.[10] Spatial separation of the chromophore-acceptor units by the protein has important consequences on the stabilization of the photogenerated redox product against back electron transfer. Furthermore, the fact that redox sites embedded in proteins lack direct electrical communication with their macroscopic environment,[11,12] suggests that a reductive photoenzyme could be coupled to an oxidative biocatalyst through a reversible diffusional electron mediator, Figure 1. Recently, we demonstrated a semisynthetic approach to prepare photoenzymes by reconstitution of proteins followed by their modification with an appropriate chromophore.[13] It was demonstrated that eosin-modified Co(II)-protoporphyrin IX reconstituted myoglobin, Eo^{2-}−Mb−Co(II), acted as photoenzyme for the photoinduced hydrogenation of acetylene to ethylene, and CO_2 fixation to formate using Na_2EDTA as sacrificial electron donor. Here we report on the cyclic coupling of the reductive semisynthetic photoenzyme, Eo^{2-}−Mb−Co(II), to the oxidative enzyme lactate dehydrogenase, LDH, using a *reversible* ferrocene electron mediator as electron shuttle between the oxidative and reductive sites.

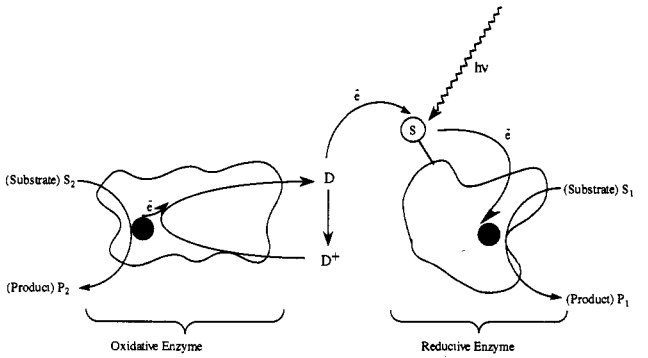

Figure 1. Scheme of cyclic photosynthetic assembly that includes a reductive photoenzyme and oxidative biocatalyst coupled by a diffusional, reversible, electron-transfer mediator.

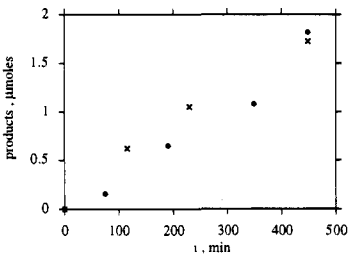

Figure 2. Rates of formation of products upon illumination of a photosystem, 3 mL, phosphate buffer, 0.1 M, pH = 7.5, that includes Eo^{2-}−Mb−Co(II), 0.11 mg·mL^{-1}, LDH, 0.6 units·mL^{-1}, **1**, 8×10^{-3} M, and lactic acid, 10^{-2} M, under an atmospheric pressure of acetylene: (●) rate of ethylene formation, (×) rate of pyruvic acid formation.

Steady-state illumination ($\lambda > 495$ nm) of a photosystem consisting of Eo^{2-}−Mb−Co(II), 0.33 mg, lactate dehydrogenase, LDH (EC 1.1.2.3), 1.8 units in 3 mL of aqueous phosphate buffer solution, pH = 7.5, that includes *N*-(methylferrocene)-caproic acid (**1**), 8×10^{-3} M, and lactic acid, 1×10^{-2} M, under an atmospheric pressure of acetylene, C_2H_2, results in the formation of ethylene and pyruvic acid.[14] Figure 2 shows the rate of C_2H_4 and pyruvic acid formation at time intervals of illumination. The reduced and oxidized products C_2H_4 and pyruvic acid, respectively, are formed at a 1:1 molar ratio, and the quantum yield of the process corresponds to $\phi = 2 \times 10^{-3}$. Control experiments reveal that all of the components are required to drive the photoinduced process and that the application of the eosin-modified reconstituted Co(II)-myoglobin is essential. Upon irradiation of photosystems that include eosin as a diffusional component, Mb−Co(II), and all other components, or Eo^{2-} and Co(II)-protoporphyrin IX and all other components, no products were detected.

Time-resolved laser flash photolysis experiments and complementary electrochemical studies demonstrate that the sequence of reactions outlined in Figure 3 leads to the cyclic photoinduced oxidation of lactate by acetylene. The primary process involves electron-transfer quenching of the eosin chromophore by the Co(II)-protoporphyrin center, eq 1, $k_q = 5.2 \times 10^4$ s^{-1}. The

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(14) Ethylene was analyzed by GC with a Poropak N. column, 40 °C, N_2 (30 mL·min^{-1}) carrier gas, FID detector. Pyruvic acid was analyzed by HPLC using an anion-exchange column (Shodex, KC-811), H_3PO_4 0.1% as eluent, UV detection $\lambda = 210$ nm. The light intensity of a 150 W Xe lamp was 10 mW·cm^{-2}.

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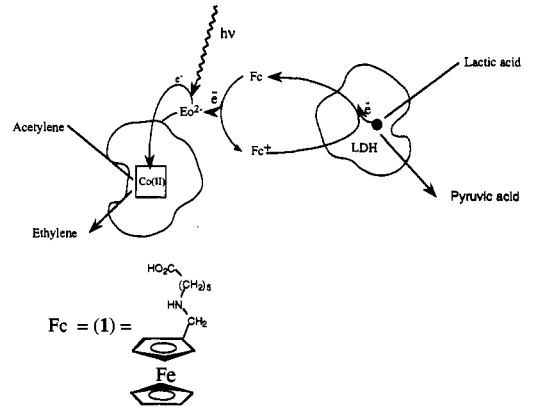

Figure 3. Scheme of electron-transfer pathway and cyclic photoinduced hydrogenation of acetylene by lactate using Eo^{2-}−Mb−Co(II) and LDH as biocatalysts.

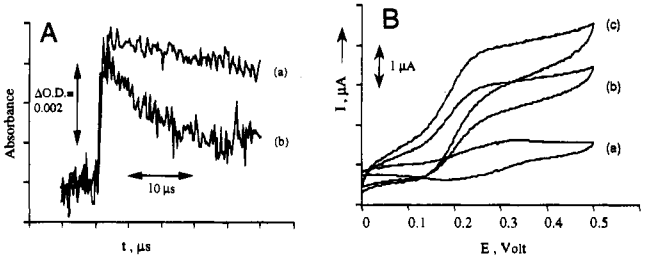

Figure 4. (A) Transient decay of the photogenerated ferrocenylium cation, 1^+ (followed at λ = 630 nm): (a) in the presence of Eo^{2-}−Mb−Co(I); (b) upon addition of LDH and lactate, 1×10^{-2} M. (B) Cyclic voltammograms representing the electrocatalytic anodic currents upon electrobiocatalyzed oxidation of lactate in the presence of LDH, 0.15 units/mL, and **1**, 3×10^{-4} M, as electron-transfer mediator: (a) 0 M lactic acid; (b) 2×10^{-3} M lactic acid; (c) 1×10^{-2} M lactic acid. All experiments were recorded in an electrochemical cell that included 0.01 M phosphate buffer, pH = 7.5, Na_2SO_4, 0.1 M, as electrolyte solution, and a Au-electrode and SCE were applied as working and reference electrodes, respectively. All experiments were performed under Ar, scan rate = 2 $mV \cdot s^{-1}$.

lifetime of the redox intermediates is 7 μs, and in the absence of the reversible ferrocene electron donor (**1**), recombination occurs, eq 2.[15] In the presence of **1**, oxidation of the electron donor by the oxidized photosensitizer takes place, eq 3. Formation of the ferrocenylium cation is evident by identification of its characteristic absorbance at λ = 630 nm. The resulting redox species exhibit an extremely long lifetime corresponding to 0.2 ms, eq 4.[16] Figure 4A curve a shows the very slow decay of the resulting ferrocenyl cation redox species. Stabilization of the redox intermediates to back electron transfer is attributed to the shielding of the reduced Co(I) by the protein from the oxidized ferrocenyl cation present as diffusional component in the aqueous medium. The generated ferrocenyl cation communicates with the LDH and mediates the oxidation of the active site and subsequent oxidation of lactate to pyruvate, eq 5. This is evident from the rapid decay of the ferrocenyl cation in the presence of LDH and lactate, Figure 4A, curve b. Electrochemical experiments provide further support that ferrocenyl cation mediates the oxidation of lactate in the presence of LDH, Figure 4B. While the ferrocene electron donor exhibits a quasi-reversible cyclic voltammogram, E° = 0.27 V vs SCE, an electrocatalytic anodic current is observed upon addition of LDH and lactate, implying that the ferrocenyl cation mediates the electrobiocatalyzed oxidation of lactate. Note that electrocatalytic oxidation of lactate is recorded at a scan rate of 2 $mV \cdot s^{-1}$, leading to the quasi-reversible voltammogram of the ferrocene component itself. No electrocatalytic oxidation of lactate LDH is observed in the absence of the ferrocene relay. Thus, the electrocatalytic anodic currents observed at the ferrocene oxidation potential with LDH and lactate in the presence of the ferrocene electron mediator imply that electrobiocatalyzed oxidation of lactate by the ferrocenyl cation occurs. The stabilization of the redox species and the mediated LDH oxidation of lactate facilitate the complementary hydrogenation of acetylene to ethylene by the intermediate Co(III) hydride species, eq 6.

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

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

$$Eo^{\bullet-}-Mb-Co(I) + Fc \xrightarrow{k_{ox} = 2 \times 10^9\ M^{-1} \cdot s^{-1}} Eo^{2-}-Mb-Co(I) + Fc^+ \quad (3)$$

$$Eo^{2-}-Mb-Co(I) + Fc^+ \xrightarrow{k_b{}^2 = 6.25 \times 10^5\ M^{-1} \cdot s^{-1}} Eo^{2-}-Mb-Co(II) + Fc \quad (4)$$

$$Fc^+ + CH_3CH(OH)-CO_2H \xrightarrow{LDH} Fc + CH_3C(O)CO_2H \quad (5)$$

$$Eo^{2-}-Mb-Co(I) \xrightarrow{H^+} Eo^{2-}-Mb-Co(III)-H \xrightarrow{C_2H_2} Eo^{2-}-Mb-Co(III) + C_2H_4 \quad (6)$$

The photosystem reveals cyclic activity, and at the final steady-state illumination conditions shown in Figure 2, the components Eo^{2-}−Mb−Co(II) and LDH exhibited 110 and 50 turnover cycles, respectively.[17] It is interesting to note that, in analogy to the present system, the photosynthetic bacteria chloroflexus employs lactate as the oxidation substrate.[18]

In conclusion, we demonstrated that the Eo^{2-}−Mb−Co(II) photoenzyme and a reversible ferrocene electron donor provided an assembly for the effective stabilization of the photogenerated redox products against recombination. This facilitated the subsequent oxidation and reduction of lactate and acetylene, respectively. The lack of electrical communication between a redox site embedded in a protein and its macroscopic environment is well-established.[12] Although substantial efforts are directed to facilitate electrical communication between redox proteins and their surroundings,[11,12] specifically in biosensor technology, we utilize this fundamental feature to stabilize photoproducts against back electron transfer. Further efforts to integrate the system into a photosynthetic device by covalent linkage of the oxidative and reductive biocatalyst are under way.

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(15) The recombination process was followed by the decay of the photogenerated Co(I) (λ = 404 nm).

(16) Ca. 80% of the photogenerated $Eo^{\bullet-}$−Mb−Co(I) is reduced by **1** to generate the ferrocenyl cation. The remaining fraction decays by intramolecular back electron transfer, eq 2. The decay of photogenerated ferrocenyl cation was followed at λ = 630 nm.

(17) The quantum yield of the photogenerated ferrocenyl cation that mediates the oxidation of lactate (or hydrogenation of acetylene) is ca. 7.2 $\times 10^{-3}$. Note, however, that for oxidation of lactate under steady-state conditions, cyclic photoreduction of Co(III) to Co(II) and of Co(II) to Co(I) is required. As the quantum yield for pyruvic acid formation is 2×10^{-3}, it implies that photoreduction of Co(III) to Co(II) proceeds with a quantum yield of ca. 0.28.

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