Furthermore, they provide the first structural and molecular dynamics information on such a catalyst–cocatalyst ion pair and suggest that the scope of effective Lewis acid cocatalysts may be considerably broader than heretofore appreciated.

Acknowledgment. We are grateful to the Division of Chemical Sciences, Office of Basic Energy Sciences, Office of Energy Research, U.S. Department of Energy, for support of this research under Grant DE-FG02-86ER13511.

Supplementary Material Available: Tables of atomic coordinates, anisotropic thermal parameters, and bond distances and angles for **2** (13 pages); listing of observed and calculated structure factors for **2** (29 pages). Ordering information is given on any current masthead page.

Electrically Wired Glutathione Reductase: A Biocatalyst for the Photochemical Reduction of Glutathione

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Received December 11, 1990

Electron transfer to (and from) redox sites of enzymes is inhibited by the insulating shell of the protein. Protein modification by electron relay components[1] and protein interaction with redox polymers[2] facilitate electron transfer to the active site by forming a "wire" that penetrates the insulating shell. Electrically wired biocatalysts (glucose oxidase and D-amino acid oxidase) were coupled to an electrode, and electron transfer was established.[1] Photosensitized electron transfer reactions in which the electron transfer between the photosystem and the active site of the enzyme is mediated by a redox polymer has recently been achieved with nitrate reductase by immobilizing the enzyme in a bipyridinium copolymer.[3] Unlike nitrate reductase, many other redox enzymes fail to establish electron transfer when immobilized in such a polymer, since the active site is shielded by a thick protein shell. This is the case with glutathione reductase. Here we report on a relay-modified glutathione reductase exhibiting electron-transfer properties. We show that the relay-modified enzyme interacts directly with excited species. We also demonstrate the improved biocatalytic performance of the relay-modified enzyme when it is immobilized in a redox polymer matrix. It should be noted that native glutathione reductase requires NAD(P)H as a cofactor for its catalytic activity. With the relay-modified enzyme, however, the cofactor is excluded and electrical wiring is maintained in the assembly.

Glutathione reductase (EC 1.6.4.2, Sigma type III from bakers' yeast) is modified by anchoring 4,4'-bipyridinium-1,1'-dipropionate,[4] PAV, to its lysine residues using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide, EDC, as the coupling agent.[5] Illumination ($\lambda > 420$ nm) of a photosystem that includes $Ru(bpy)_3^{2+}$ as a photosensitizer, EDTA as a sacrificial electron donor, and PAV-modified glutathione reductase in the presence of oxidized glutathione, GSSG, results in the formation of reduced glutathione,[6] GSH. Figure 1A shows the evolution of GSH in photosystems that contain the modified enzyme at varying degrees of relay loadings.[7,8] It is evident that the higher the relay loading, the faster the reaction rate. Control experiments reveal that the biocatalyzed transformation does not occur in the dark, nor does it occur in the absence of any of the components constituting the photosystem. Laser flash photolysis experiments, following the excited-state lifetime of $Ru(bpy)_3^{2+}$ at different PAV-modified

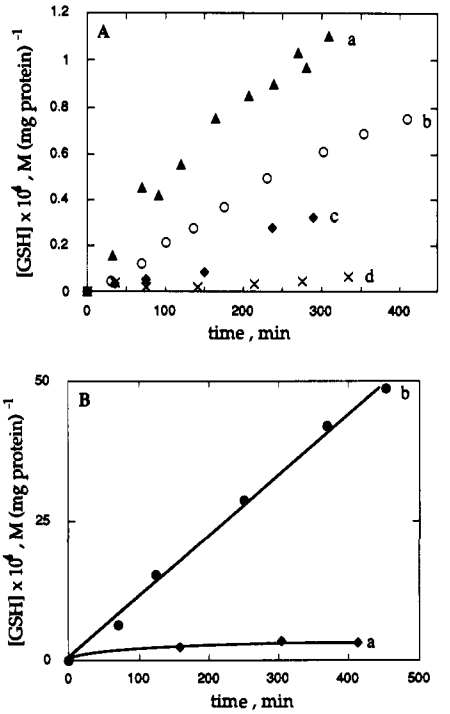

Figure 1. (A) Rate of GSH evolution (normalized per milligram of protein) under illumination ($\lambda > 420$ nm) in a photochemical system consisting of $[Ru(bpy)_3^{2+}] = 6.8 \times 10^{-5}$ M, [EDTA] = 0.01 M, [GSSG] = 0.01 M in 3 mL of 0.1 M phosphate buffer, pH 7.5. The loading degrees of PAV on the enzyme (mole/mole) in the various systems are (a) 3.9, (b) 1.8, (c) 1.4, and (d) 0.5. (B) Rate of GSH evolution (normalized per milligram of protein) in the photosystem described in part A: (a) in the system described in graph Aa; (b) in a photosystem composed of 1.66 g of the redox polymer which contains the immobilized relay-modified glutathione reductase.

(1) (a) Degani, Y.; Heller, A. *J. Am. Chem. Soc.* **1988**, *110*, 2615. (b) Heller, A. *Acc. Chem. Res.* **1990**, *23*, 128.
(2) (a) Degani, Y.; Heller, A. *J. Am. Chem. Soc.* **1989**, *111*, 2357. (b) Gregg, B. A.; Heller, A. *Anal. Chem.* **1990**, *62*, 258. (c) Gorton, L.; Karan, H. I.; Hale, P. D.; Inagaki, T.; Okamoto, Y.; Skotheim, T. A. *Anal. Chim. Acta* **1990**, *228*, 23. (d) Hale, P. D.; Inagaki, T.; Lee, H. S.; Karan, H. I.; Okamoto, Y.; Skotheim, T. A. *Anal. Chim. Acta* **1990**, *228*, 31.
(3) Willner, I.; Riklin, A.; Lapidot, N. *J. Am. Chem. Soc.* **1990**, *112*, 6438.
(4) Le Berre, A.; Delacroix, A. *Bull. Soc. Chim. Fr.* **1973**, *7–8*, 2404.
(5) A typical experiment involves dissolving 4.0 mg of PAV, 160 mg of HEPES (sodium salt), and 360 mg of urea in 3.2 mL of water. The solution is brought to pH 7.0 by adding 1 M HCl. Then 1 mL of the resulting solution is mixed with 1 mL of 0.05 M HEPES buffer solution, pH 7.1, containing 11.2 mg of the enzyme, and 13.3 mg of EDC is added. The reaction is stirred at 0 °C for 3 h, and the solution is dialyzed extensively against 0.1 M NaH_2PO_4 buffer, pH 7.5, until the enzyme solution does not contain any free PAV. The resulting solution contains 5.0 mg/mL of protein (assayed by the Biuret method), and the concentration of the protein-bound PAV is estimated to be 1.7×10^{-4} M, corresponding to 3.9 molecules of bound PAV/enzyme molecule. When lower loading values of the relay are desired, the concentration of PAV in the reaction mixture and the quantity of the coupling agent are decreased.
(6) The concentration of GSH is determined by Ellman's method; 1.9 mL of 0.1 M phosphate buffer, pH 7.6, and 0.1 mL of 5,5'-dithiobis(2-nitrobenzoic acid), BIS, reagent (1.6 mg of BIS/mL in 0.1 M phosphate buffer at pH 7.0) are added to 0.1 mL of the reaction sample. Absorbance at $\lambda = 412$ nm ($\epsilon = 13\,600$ M^{-1} cm^{-1}) is measured after 2 min; cf.: McNiel, T. L.; Beck, L. V. *Anal. Biochem.* **1986**, *22*, 431.
(7) The concentration of the protein-bound PAV in the solution is estimated by the following method: 0.2 mL of the sample is diluted with 0.8 mL of 0.1 M phosphate buffer, pH 7.5. The solution is deaerated, and 5 mg of sodium dithionite is dissolved in it. The absorption at $\lambda = 602$ nm ($\epsilon = 11\,800$ M^{-1} cm^{-1}) is measured after 5 min of further deaeration. The loading corresponds to the mole ratio of bound PAV to that of the protein; the molecular weight of glutathione reductase is taken as 118 000; cf.: Colman, R. F. *Methods Enzymol.* **1971**, *17B*, 500.
(8) The PAV-modified glutathione reductase exhibits 72% of the activity of the native enzyme. The assay for this comparison consists of a photochemical system that includes $Ru(bpy)_3^{2+}$, 6.8×10^{-5} M, MV^{2+}, 1×10^{-2} M, GSSG, 1×10^{-2} M, and EDTA, 1×10^{-2} M, pH 7.5. Native glutathione reductase or the modified enzyme, 1.85 mg, is introduced into the system, and the formation of GSH is followed.

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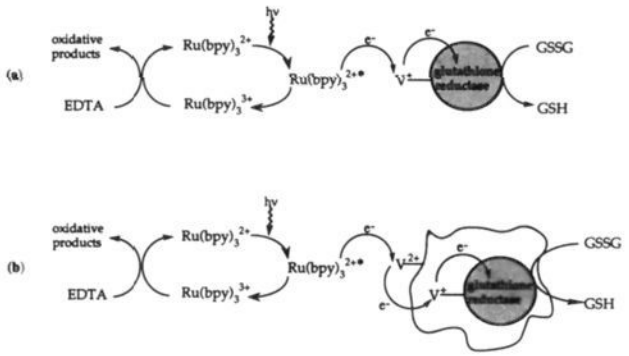
Scheme I. Schematic Representation of Electrical Wiring of Glutathione Reductase in the Photochemical Assembly[a]



[a] (a) Electrical wiring of the enzyme and direct coupling to the photochemical process. (b) Immobilization of the relay-modified enzyme in a redox polymer and coupling of the enzyme–polymer wired assembly to the photosystem.

glutathione reductase concentrations, allow us to evaluate the quenching rate constant, k_q. The derived quenching rate constant corresponds to $k_q = 5.6 \times 10^9\ M^{-1}\ s^{-1}$, indicating that the modified enzyme effectively quenches the excited state of the sensitizer. We therefore conclude that the reduction of GSSG proceeds through the cycle shown in Scheme I, part a. Quenching of excited $Ru(bpy)_3^{2+}$ by the protein-bound PAV generates the reduced PAV and the oxidized sensitizer. Secondary electron transfer through PAV moieties to the active site of glutathione reductase enables the reduction of GSSG to GSH. The light-harnessing compound is recycled through the oxidation of EDTA by the oxidized sensitizer.

There are two possible reasons for the aforementioned relationship between the PAV loading degree of glutathione reductase and the observed reaction rate: (i) If the effectiveness of electrical wiring is the factor determining the reaction rate, lowering the relay loading on the protein could lead to a lower reaction rate. (ii) The biocatalyzed process may be controlled by the primary photosensitized electron transfer process, and the rate of the latter process depends directly on the concentration of the quencher. Knowing the quenching rate constant of excited $Ru(bpy)_3^{2+}$ by PAV–glutathione reductase allows us to estimate the fraction of $Ru(bpy)_3^{2+}$ fluorescence quenched by the enzyme with varying relay loadings. A plot of the observed reaction rate of the photosystem (Figure 1A) against the fraction of excited species being quenched gives a linear relationship. We therefore deduce that the rate-limiting step in the photosystem is the primary electron transfer quenching of the excited species.

This would suggest that immobilizing the modified enzyme in a redox polymer, which contains a high concentration of the quencher, could be advantageous, as it would accelerate the reaction rate. Immobilization can also stabilize the enzyme against degradation and is beneficial with respect to enzyme recycling possibilities.[9] The relay-modified enzyme has been, therefore, immobilized in a redox copolymer composed of acrylamide and 1-methyl-1′-(3-acrylamidopropyl)-4,4′-bipyridinium.[3] Indeed, the observed reaction rate of the photosensitized reduction of GSSG, when the enzyme is immobilized in the redox polymer, is ca. 25 times faster than in the homogeneous system, as can be seen in Figure 1B. This is attributed to efficient electron-transfer quenching of the excited sensitizer by the relay moieties of the polymer. The reduced polymer serves as an electron reservoir for the relay-modified enzyme which is trapped inside it, hence the higher overall reaction rate. The electrons are transferred from the reduced polymer to the protein-bound PAV groups, which in turn transfer the electrons to the active site of the enzyme, as in the homogeneous system. It should be noted that the native enzyme was unable to accept electrons from this redox polymer upon immobilization. The electron transfer must be mediated by the protein-bound PAV. The process with the immobilized enzyme is summarized in Scheme I, part b. The stabilizing effect of the polymer on the enzyme is also evident from Figure 1B. In the homogeneous system the activity levels off after ca. 3 h, due to the degradation of the enzyme, whereas the immobilized enzyme exhibits prolonged catalytic activity with no apparent sign of degradation.

In conclusion, the chemical modification of glutathione reductase with electron relays has proved to be an effective way of electrically wiring the enzyme. The modification facilitates electron transfer between the enzyme and its environment, thus enabling the photochemical reduction of GSSG to GSH and eliminating the need for the natural cofactors of this enzyme. Immobilization of the electrically wired protein in a redox copolymer matrix results in a multicomponent wired assembly, where the polymer acts as an electron reservoir.

Acknowledgment. The research project is supported by the Bundesministerium für Forschung und Technologie, Germany (BMFT), and by the Ministry of Science and Technology, Israel (scholarship to N.L.). N.L. acknowledges the support of the Ben Gurion Fund.

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Additions and Corrections

Effects of a Polarizable Medium on the Charge-Transfer States of the Photosynthetic Reaction Center from *Rhodopseudomonas viridis* [*J. Am. Chem. Soc.* **1990**, *112*, 7828–7830]. MARK ALAN THOMPSON and MICHAEL C. ZERNER*

Page 7829: The first sentence in ref 23 should read as follows: In calculations of the (bare) BChlb dimer alone that incorporate 785 configurations, we calculate the energy of Qy1 and Qy2 as 10 785 and 12 345 cm^{-1}, respectively.