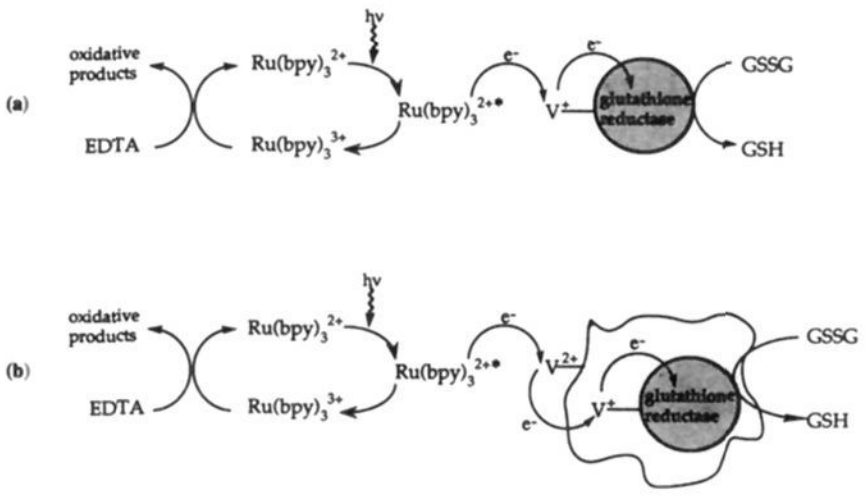
Scheme I. Schematic Representation of Electrical Wiring of Glutathione Reductase in the Photochemical Assembly[a]



[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.

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(9) Ramachandran, P. A.; Krishna, R.; Panchal, C. B. *J. Appl. Chem. Biotechnol.* **1976**, *26*, 214.

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.