Amperometric Transduction of Optical Signals Recorded by Organized Monolayers of Photoisomerizable Biomaterials on Au Electrodes

Mazzi Lion-Dagan, Eugenii Katz, and Itamar Willner*

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

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Application of photoswitchable biological substances as information storage and bioelectronic materials is a subject of extensive research effort.[1] Modification of biomaterials such as emzymes[2] or receptor proteins[3] by reversibly photoisomerizable compounds has led to "on-off" photoswitchable biocatalytic activities of enzymes and photostimulated binding of substrates by proteins. Also, immobilization of enzymes in photoisomerizable polymer membranes provides a means to activate/deactivate the biocatalysts by controlling the substrate permeabilities across the photoisomer states of the polymers.[4] In all these systems, the photoswitchable properties of the enzymes are detected by the accumulation of products as a function of time. Hence, the recording of the optical signals by the photoswitchable biomaterials is not instantaneously transduced as a physical signal, and the systems lack the organization needed to function as bioelectronic devices. Recently, we organized enzymes,[5] i.e., glutathione reductase or glucose oxidase, and native cofactors such as pyrroloquinolinoquinone[6] (PQQ) in self-assembled monolayers (SAMs) on electrode surfaces. Here we wish to report on the organization of photoisomerizable glucose oxidase (GOD) monolayers and PQQ monolayers on Au electrodes. These assemblies allow the reversible recording of optical signals and their amperometric transduction.

PQQ monolayers act as an electrocatalytic interface for the oxidation of NAD(P)H in the presence of Ca^{2+}.[6a] We have organized a mixed SAM consisting of PQQ and photoisomerizable spiropyran units on Au electrodes by the sequence of reactions outlined in Scheme 1. The ratio of PQQ/spiropyran units corresponds to ca. 1:1.[7] Figure 1 (top) shows the cyclic voltammograms of the PQQ–spiropyran monolayer alone (curve a) and those of the monolayer electrode in the two photoisomer states, the spiropyran (SP) state (generated by illumination, $\lambda > 475$ nm), curve b, and the merocyanine (MR) state (generated by illumination, $360 < \lambda < 380$ nm), curve c, in the presence of 25 mM NADPH and 10 mM Ca^{2+}. In the SP monolayer state, effective electrocatalyzed oxidation of NADPH proceeds, while in the MR monolayer state, the process is inhibited. Previous studies[8] have indicated that upon photoisomerization of spiropyran-modified electrodes to the respective positively charged

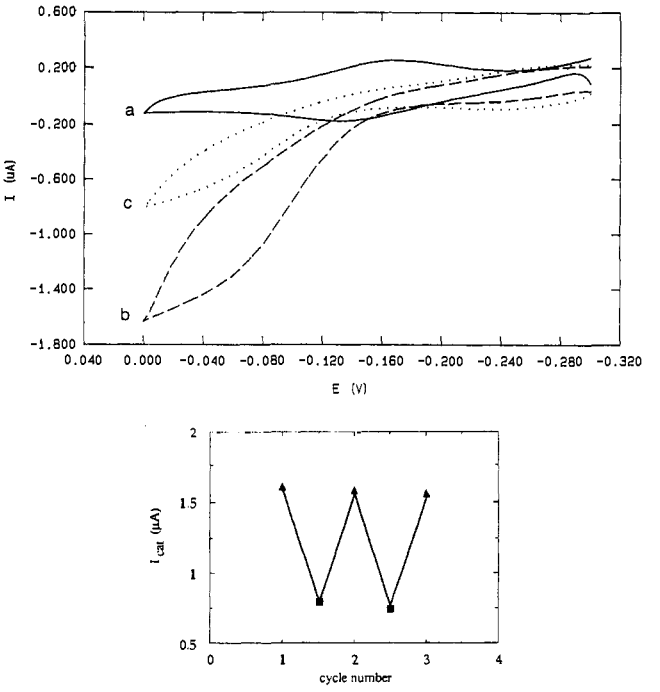

Figure 1. (Top) Cyclic voltammograms corresponding to NADPH oxidation by PQQ–spiropyran mixed monolayer electorde: (a) without NADPH; (b) electrode in SP state obtained after 5 min of electrode illumination, $\lambda < 475$ nm; (c) electrode in MR state obtained after 2 min of illumination $360 < \lambda < 380$ nm. Electrolyte solution is composed of 0.1 M Na_2SO_4 and 0.01 M phosphate buffer, pH = 7, [NADPH] = 2.5×10^{-2} M, and $[Ca^{2+}] = 1 \times 10^{-2}$ M, scan rate, 1 $mV \cdot s^{-1}$. (Bottom) Cyclic amperometric responses of PQQ–spiropyran monolayer electrode obtained upon electrocatalyzed oxidation of NADPH: (▲) electrode in SP state; (■) electrode in MR state.

protonated merocyanine isomer, the electrode potential is positively shifted. Thus, the photoswitchable electrocatalyzed oxidation of NADPH by the PQQ–spiropyran monolayer electrode is attributed to different interactions of the Ca^{2+} cocatalyst with the photoisomerizable monolayer assembly. In the spiropyran isomer state, Ca^{2+} is associated with PQQ and electrocatalyzed oxidation of NADPH occurs. Upon photoisomerization to the positively charged protonated merocyanine, Ca^{2+} is repelled from the monolayer, and the electrocatalyzed transformation is inhibited. The photostimulated electrocatalytic properties of the PQQ–spiropyran monolayer electrode allow the reversible transduction of amperometric signals as a result of cyclic optical activation/deactivation of the PQQ monolayer, Figure 1 (bottom). Here, photoisomerization of the monolayer to the SP state ($\lambda > 475$ nm) results in a high amperometric response, while further illumination to the MR monolayer ($360 < \lambda < 380$ nm) is reflected by a decrease of the current being developed in the electrochemical cell.

A further example for amperometric transduction of photostimulated activation/deactivation of biomaterials was developed using photoisomerizable glucose oxidase as active monolayer. The GOD was chemically modified by **1**. The loading of GOD by spiropyran units corresponds to 21.[9] SP-modified GOD (SP-GOD) exhibits photoisomerizable properties,[10] and, upon illumination ($360 < \lambda < 380$ nm), it photoisomerizes to MR-GOD. Further irradiation of the latter isomer, $\lambda > 475$ nm, results in the formation of SP-GOD. The spiropyran-modified

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(2) (a) Willner, I.; Rubin, S.; Riklin, A. *J. Am. Chem. Soc.* **1991**, *113*, 3321. (b) Aizawa, M.; Namba, K.; Suzuki, S. *Arch. Biochem. Biophys.* **1977**, *182*, 305.

(3) (a) Willner, I.; Rubin, S.; Wonner, J.; Effenberger, F.; Bäuerle, P. *J. Am. Chem. Soc.* **1992**, *114*, 3150. (b) Willner, I.; Rubin, S.; Cohen, Y. *J. Am. Chem. Soc.* **1993**, *115*, 4937.

(4) (a) Willner, I.; Rubin, S.; Zor, T. *J. Am. Chem. Soc.* **1991**, *113*, 4013. (b) Willner, I.; Rubin, S.; Shatzmiller, R.; Zor, T. *J. Am. Chem. Soc.* **1993**, *115*, 8690.

(5) (a) Willner, I.; Katz, E.; Riklin, A.; Kasher, R. *J. Am. Chem. Soc.* **1992**, *114*, 10965. (b) Willner, I.; Lapidot, N.; Riklin, A.; Kasher, R.; Zahavy, E.; Katz, E. *J. Am. Chem. Soc.* **1994**, *116*, 1428. (c) Willner, I.; Riklin, A.; Shoham, B.; Rivenzon, D.; Katz, E. *Adv. Mater.* **1993**, *5*, 912.

(6) (a) Katz, E.; Lötzbeyer, T.; Schlereth, D. D.; Schuhmann, W.; Schmidt, H.-L. *J. Electroanal. Chem.*, in press. (b) Willner, I.; Riklin, A. *Anal. Chem.* **1994**, *66*, 1535.

(7) The PQQ/spiropyran ratio was estimated by subtraction of the charge associated with the reduction of PQQ in the mixed monolayer from the value corresponding to a fully covered PQQ monolayer electrode.

(8) (a) Kato, S.; Aizawa, M.; Suzuki, S. *J. Membr. Sci.* **1976**, *1*, 289. (b) Hasebe, Y.; Anzai, J.; Ueno, A.; Osa, T.; Chen, C. *J. Phys. Org. Chem.* **1988**, *1*, 309.

(9) GOD includes 54 lysine residues. The loading of spiropyran units associated with GOD was estimated by following the absorption spectrum of the MR-GOD solution ($\lambda = 536$ nm, $\epsilon = 2300$ M^{-1} cm^{-1}) that includes a protein content corresponding to 1.86 $mg \cdot mL^{-1}$.

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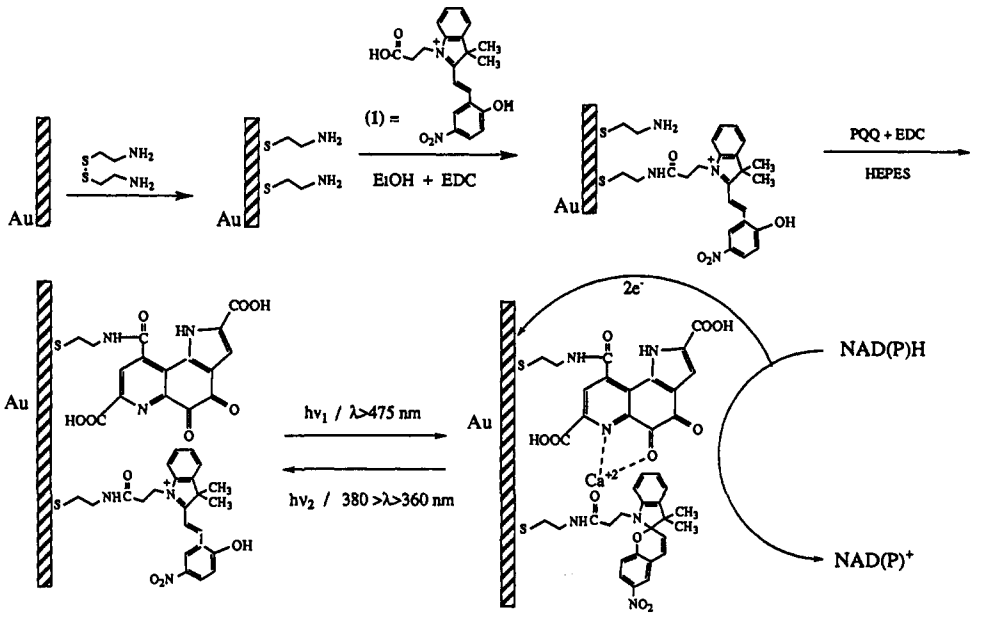
Scheme 1



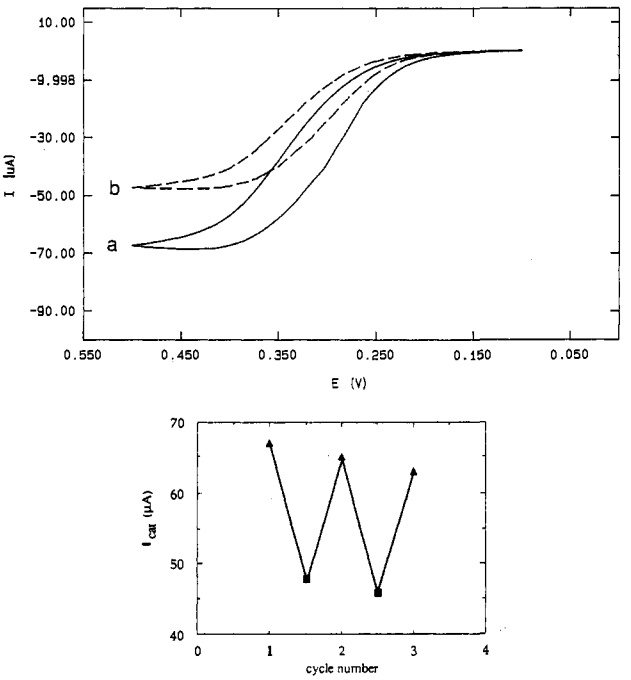
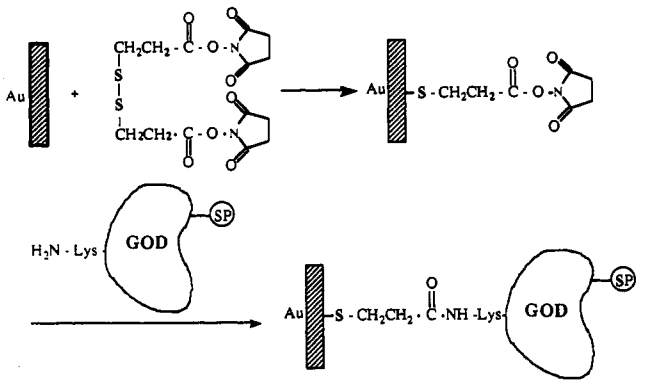


Figure 2. (Top) Cyclic voltammograms for the electrobiocatalyzed oxidation of glucose, 2.5×10^{-2} M, by the photoisomerizable GOD monolayer electrode: (a) electrode in GOD-SP state; (b) electrode in GOD-MR state. Electrolyte solution is composed of 0.1 M Na_2SO_4 and 0.01 M phosphate buffer, pH = 7.0, that contains ferrocene carboxylic acid, 5×10^{-3} M as electron mediator; scan rate, 5 $mV \cdot s^{-1}$. (Bottom) cyclic amperometric responses of the photoisomerizable GOD monolayer electrode: (▲) electrode in GOD-SP state; (■) electrode in GOD-MR state.

GOD reveals photoswitchable activities in a homogeneous phase.[11] The SP-GOD was immobilized as a SAM onto an Au electrode as outlined in Scheme 2. The amperometric response of the SP-

Scheme 2



GOD monolayer electrode (formed upon irradiation, $\lambda > 475$ nm) using ferrocene carboxylic acid, 5 mM, as electron transfer mediator and glucose, 25 mM, as substrate[12] is shown in Figure 2 (top, curve a). Illumination of the electrode, $360 < \lambda < 380$ nm, generates the MR-GOD monolayer electrode, and a substantially lower amperometric signal is observed (curve b). Upon cyclic photoisomerization of the enzyme monolayer electrode between the states SP-GOD and MR-GOD, the biocatalyst is reversibly activated and deactivated. The photoswitchable activities of the biocatalyst are transduced as amperometric signals, Figure 2 (bottom). Effective electrocatalyzed oxidation of glucose proceeds by the SP-GOD monolayer electrode as reflected by the high amperometric response of the system, while the electrobiocatalyzed transformation is inhibited in the presence of the MR-GOD monolayer electrode.[13]

We thus conclude that organization of photoisomerizable electroactive biomaterials as SAM on electrodes provides a means to transduce the light-stimulated on–off activities of the respective biomaterials by an amperometric signal.

Acknowledgment. This research was supported by a grant from the Ministry of Science and Technology, Israel, and the Commission of the European Communities.

(10) For general reviews on photoisomerization of spiropyrans, see: (a) Guglielmetti, R. In *Photochromism: Molecules and Systems*; Dürr, H., Bouas-Laurent, H., Eds.; Elsevier: Amsterdam, 1990; p 314. (b) Bertelson, R. C. In *Techniques of Chemistry. Volume 3, Photochromism*; Brown, G. H., Ed.; Wiley: New York, 1971; p 45.

(11) The SP-GOD reveals a 2-fold higher activity as compared to MR-GOD in solution. The photoswitchable activities in solution were determined by following the oxygenic biocatalyzed oxidation of glucose by the SP-GOD and MR-GOD enzymes. The generated H_2O_2 was colorimetrically analyzed with *o*-dianisidine.

(12) Cass, A. E. G.; Davis, G.; Francis, G. D.; Hill, H. A. O.; Aston, W. J.; Higgins, I. J.; Plotkin, E. V.; Scott, L. D. L.; Turner, A. P. F. *Anal. Chem.* **1984**, *56*, 667.

(13) The slight decrease in the amperometric responses of the SP-GOD and MR-GOD upon cyclic irradiation of the electrode is attributed to ca. 4% denaturation of the enzyme by the UV irradiation step.