Application of Photoisomerizable Antigenic Monolayer Electrodes as Reversible Amperometric Immunosensors

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Antibody–antigen interactions play a major role in biosensor technology.[1,2] The strong antigen–antibody interactions limit the applicability of antigen (or antibody) sensing surfaces to single-cycle analytic devices. Conversion of single-cycle immunosensors to multicycle devices is expected to provide a scientific and technological breakthrough in biosensor technology. Here we report on the development of amperometric immunosensors by the organization of antigenic self-assembled monolayer electrodes and on the use of photoisomerizable antigen monolayer electrodes as an approach to develop reversible amperometric immunosensors. Modification of biomaterials by photoisomerizable units[3] provided a means to photostimulate "on-off" biocatalytic and binding properties of enzymes[4] and proteins.[5] Photoregulated binding a *trans*-azobenzene hapten to the respective antibody[6a] and selective association of a dinitro spiropyran with the anti-dinitrophenyl (anti-DNP) antibody[6b] have revealed selective association of photoisomerizable antigens with their antibodies.[6] Development of amperometric biosensors has been examined extensively in recent years,[7] and redox modified enzyme monolayer electrodes were applied as amperometric sensors.[8,9]

The development of the amperometric biosensor is based on the construction of a self-assembled antigen monolayer on the electrode surface. This electrode electrically communicates with a redox probe, R^+/R, in the electrolyte solution and provides an amperometric response. Interaction of the electrode with the antibody, Ab, results in binding of the Ab to the monolayer. The electrode surface is then insulated toward the solubilized redox probe, and its amperometric response decreases. The extent of electrode insulation is controlled by the Ab concentration and the time of incubation of the monolayer electrode with the Ab solution. Figure 1 shows the stepwise construction[8] of the antigen monolayer toward the DNP-Ab. The amperometric response of the antigen monolayer electrode in an electrochemical cell that included $K_3Fe(CN)_6$ (**1**) as redox probe was recorded at different DNP-Ab concentrations. The difference between the amperometric signal of the antigen electrode and that of the antigen electrode in the presence of DNP-Ab, Δi_{pc}, increased linearly as

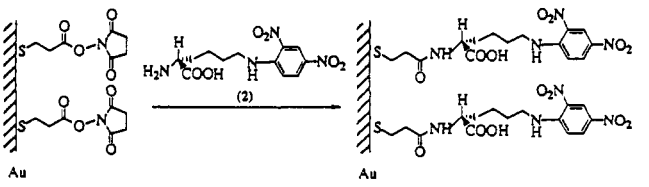

Figure 1. Organization of a dinitrophenyl antigen monolayer on an Au electrode.

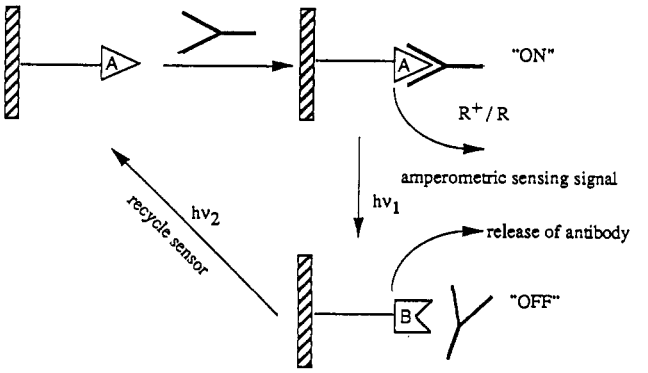

Figure 2. Schematic configuration of a reversible amperometric immunosensor utilizing a photoisomerizable monolayer electrode.

the DNP-Ab concentration was elevated, and Ab content as low as 0.5 μM could be detected by this monolayer antigen electrode.[10]

The principle of reversible amperometric immunosensors is shown in Figure 2. The antigen monolayer consists of a photoisomerizable component. In one photoisomer state (state A), the antigen recognizes the Ab, and the difference in the amperometric responses of the Ab-linked electrode and the antigen monolayer electrode, lacking the Ab, toward the solubilized redox couple provides a quantitative measure for the Ab concentration. Upon completion of the measuring cycle the antigen monolayer is photoisomerized to state B. This leads to distortion of the antigen monolayer, and the perturbed surface lacks affinity toward the Ab. The Ab is washed off and the monolayer electrode is further illuminated ($h\nu_2$) to restore the active antigen monolayer electrode. Thus, a two-step illumination procedure regenerates the active antigen electrode.

Transformation of the DNP-Ab amperometric immunosensor into a reversible multicycle sensing device was accomplished by organization of a photoisomerizable dinitrophenyl spiropyran antigen monolayer electrode, Scheme 1. The surface density of the dinitro spiropyran residues corresponds to 5.0×10^{-11} mol·cm^{-2}. The resulting **4a** dinitro spiropyran monolayer electrode exhibits reversible photoisomerizable properties.[12] Illumination of the **4a** monolayer electrode, λ = 360–380 nm, results in the **4b** monolayer electrode, and further irradiation of the **4b** monolayer electrode, λ > 495 nm, restores the **4a** monolayer. The amperometric response of **4a** monolayer electrodes in the presence

(1) Scheller, F.; Schubert, F. *Techniques and Instrumentation in Analytical Chemistry, Vol. 11: Biosensors*; Elsevier: Amsterdam, 1992; pp 275–284.
(2) Anglister, J.; Jacob, C.; Assulin, O.; Ast, G.; Pinkser, R.; Arnon, R. *Biochemistry* **1988**, *27*, 717.
(3) Willner, I.; Willner, B. In *Bioorganic Photochemistry—Biological Applications of Photochemical Switches*; Morrison, H., Ed.; Wiley: New York, 1993; pp 1–110.
(4) Willner, I.; Rubin, S.; Riklin, A. *J. Am. Chem. Soc.* **1991**, *113*, 4013.
(5) Willner, I.; Rubin, S.; Wonner, J.; Effenberger, F.; Bäuerle, P. *J. Am. Chem. Soc.* **1992**, *114*, 3150.
(6) (a) Harada, M.; Sisido, M.; Hirose, J.; Nakanishi, M. *FEBS Lett.* **1991**, *286*, 6. (b) Willner, I.; Blonder, R.; Dagan, A. *J. Am. Chem. Soc.* **1994**, *116*, 3121.
(7) (a) Wilson, G. *Biosensors: Fundamentals and Applications*; Turner, A., Karube, I., Wilson, G., Eds.; Oxford Univ. Press: New York 1987. (b) Heller, A. *Acc. Chem. Res.* **1990**, *23*, 128.
(8) Willner, I.; Katz, E.; Riklin, A.; Kasher, R. *J. Am. Chem. Soc.* **1992**, *114*, 10965.
(9) Willner, I.; Riklin, A.; Shoham, B.; Rivenzon, D.; Katz, E. *Adv. Mater.* **1993**, *5*, 912.
(10) The antigen monolayer electrode also provides a means to analyze the antigen itself. Challenging of the monolayer electrode with an analyte solution consisting of a constant concentration of the DNP-Ab and variable concentrations of antigen **2** results in competitive association of the Ab to the monolayer electrode. At high analyte concentrations of **2** the monolayer electrode is not insulated due to binding of the Ab to the antigen analyte. Using this approach, the monolayer antigen electrode was sensitive enough to detect up to 0.5×10^{-6} M of the antigen (**2**).
(11) For determination of surface coverage, cf.: Katz, E.; Solov'ev, A. A. *J. Electroanal. Chem.* **1990**, *291*, 171.
(12) A closely related monolayer of dinitro spiropyran was prepared by covalent linkage of **3** to a monolayer generated by reaction of (3-aminopropyl)-triethoxysilane to an ITO transparent electrode. The resulting spiropyran monolayer exhibits the characteristic reversible photoisomerizable properties that were followed spectroscopically. The electrochemical properties of the ITO electrode toward the DNP-Ab are similar to those described for the Au electrode but are less accurate due to their variable surface areas. Accordingly, we preferred to report the results on Au electrodes that exhibit constant surface areas.

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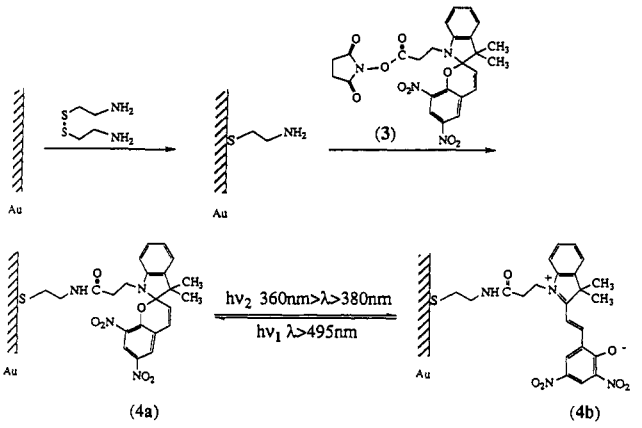
Scheme 1. Organization of a Photoisomerizable Dinitro Spiropyran Antigen Monolayer Electrode



of DNP-Ab is shown in Figure 3. The **4a** monolayer electrode is insulated by the antibody and reveals affinity toward DNP-Ab. The **4b** monolayer electrode does not interact with the DNP-Ab, and the amperometric response of the electrode is not altered in the presence of the Ab. Thus, the dinitrophenyl unit included in the **4a** spiropyran monolayer electrode acts as antigen for the DNP-Ab, while the **4b** merocyanine monolayer lacks antigen properties. Figure 3 (inset) shows the reversible activity of the dinitro spiropyran monolayer electrode as amperometric sensor for the DNP-Ab. The experiment is initiated with the **4b** monolayer electrode. The amperometric response in the presence of the DNP-Ab is similar to that of the electrode in the absence of the DNP-Ab. The high amperometric response implies that the Ab does not associate with the monolayer electrode. Illumination of the **4b** monolayer electrode, $\lambda > 495$ nm, results in the **4a** monolayer electrode.[13] Incubation of the **4a** monolayer electrode with the DNP-Ab (8 nM, 15 min) results in a decrease in the amperometric signal of the electrode, implying association of the DNP-Ab with the electrode. Further irradiation, λ = 360–380 nm, of the **4a** monolayer electrode that was interacted with the DNP-Ab results in the **4b** monolayer electrode. The rinsed electrode yields a high amperometric response, indicating that the DNP-Ab that was originally associated with the antigenic monolayer electrode was washed off. This amperometric response is not altered upon addition of the DNP-Ab. Subsequent photochemical reisomerization of the **4b** monolayer to the **4a** monolayer electrode results in a low amperometric signal upon interaction of the resulting electrode with the DNP-Ab. Thus, the activity of the monolayer electrode toward sensing the DNP-Ab is regenerated, and the two-step illumination of the photoisomerizable antigen monolayer electrode enables its reversible cyclic performance.[14]

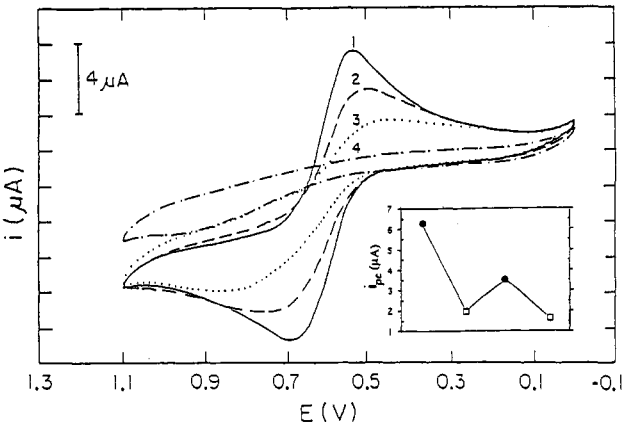


Figure 3. Cyclic voltammograms of the **4a** monolayer electrode in the presence of anti-DNP antibody, 10 μM, at different times of electrode treatment: (1) after 0 min, (2) after 3 min, (3) after 11 min, and (4) after 16 min. The **4a** electrode was produced by continuous irradiation at $\lambda > 495$ nm. (Inset) Cyclic amperometric responses of the **4**-modified monolayer electrode in the presence of anti-DNP antibody, 1.5 μM: (●) **4b** monolayer electrode; (□) **4a** monolayer electrode. The configuration of the **4b** monolayer electrode was generated by illumination of the **4a** monolayer electrode at $360 < \lambda$ 380 nm. The configuration of the **4a** monolayer electrode was produced by illumination of the **4b** monolayer electrode at $\lambda > 495$ nm. All experiments were recorded in an electrochemical cell that consisted of PBS buffer and 1.1 mM $K_3[Fe(CN)_6]$ (37 ± 2 °C), the antigen monolayer electrode (3×10^{-2} cm^2) as working electrode, Pt wire as counter electrode, and Ag/AgCl as reference electrode.

We conclude that antigen monolayer electrodes provide a sensitive interface for amperometric detection of the complementary antibody.[15] Photoisomerizable antigenic monolayers highlight a general means for cyclic operation of amperometric immunosensors.

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(13) The amperometric response of the photogenerated **4a** monolayer electrode in the absence of the DNP-Ab is identical to that of the **4b** monolayer electrode.

(14) It should be noted that only ca. 50% of the original amperometric response is restored, implying only partial dissociation of the DNP-Ab. This is attributed to incomplete isomerization of the **4a** monolayer to **4b** monolayer electrode. The electrode revealed eight reversible cycles of similar amperometric responses observed in the first cycle (50% of the initial value). Prolonged irradiation of the **4a** monolayer electrode increased the conversion yield to **4b** monolayer, and higher amperometric responses were detected due to enhanced release of the DNP-Ab. Prolonged irradiation of the electrode, however, acts adversely on the cyclic activity of the electrode, presumably due to partial decomposition of the dinitro spiropyran monolayer.

(15) Israel Patent Application No. 108726, filed on Feb 22, 1994.