Photostimulation of dinitrospiropyran-modified glucose oxidase in the presence of DNP-antibody-A biphase-switch for the amperometric transduction of recorded optical signals

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A dinitrospiropyran-modified glucose oxidase monolayer electrode in the presence of DNP-Ab acts as a biphasic switch for amperometric transduction of recorded optical signals.

Development of bioelectronic and optoelectronic devices is a rapidly advancing area at the interface of chemistry and biology.[1] Photostimulation of biomaterials to 'ON–OFF' states provides a means to record optical signals and transduce the recorded information as a biological function.[2] Photostimulation of redox enzymes has been reported as a general method for the amperometric transduction and amplification of recorded optical signals. Nitrospiropyran-modified glucose oxidase[3] and reconstituted glucose oxidase with a semisynthetic nitrospiropyran-modified FAD cofactor[4] were applied as photoswitchable enzymes for the amperometric transduction of recorded optical signals. Application of photoisomerizable electron mediators provides a further means to photostimulate the electrical communication of redox enzymes, *i.e.* glucose oxidase or glutathione reductase with electrodes.[5] An alternative approach to control bioelectrocatalysed reactions used electrodes modified by photoisomerizable monolayers.[6] In these systems, the electrical interactions of the redox proteins with the electrode were controlled by the photoisomerizable monolayer. Antigen–antibody association was similarly controlled by light signals and reversible amperometric immunosensors were organized using photoisomerizable antigen monolayer electrodes.[7]

For further development of optoelectronic devices, it is desirable to assemble more complex optical switches that mimic gate mechanisms in computer technology. In the present report we describe the photostimulated activities of dinitrospiropyran-modified glucose oxidase in the presence of the anti-dinitrophenyl antibody, DNP-Ab.

Glucose oxidase, (100 mg, from *Aspergilus niger*, E.C.1.1.3.4) was treated with *N*-propionyl-2,4-dinitrospiropyran (32 mg) in the presence of urea (180 mg), sulfo-*N*-hydroxy succinimide (10 mg) and EDC (24 mg) in a HEPES buffer solution, pH 7.5 (3.5 ml) at 4 °C for 20 h. The resulting protein was dialysed against a sodium phosphate buffer solution, pH 7.5, and further purified by elution through a Sephadex G-15 column. The loading of the resulting dinitrospiropyran-modified glucose oxidase (SP-GOD) was determined spectroscopically and corresponds to 17. The modified enzyme exhibits reversible photoisomerizable properties. Irradiation of SP-GOD, $320 < \lambda < 360$ nm, results in the protonated dinitromerocyanine-modified glucose oxidase (MRH^+-GOD) (λ_{max} = 550 nm). Further illumination of MRH^+-GOD, $\lambda > 495$ nm, regenerates SP-GOD. The activities of the two photoisomer states of modified GOD were assayed and compared to native GOD. The SP-GOD enzyme exhibits 65% of the native enzyme activity. SP-GOD revealed a 1.5-times higher activity than MRH^+-GOD in the biocatalysed oxidation of glucose by O_2. The photoisomerizable SP-GOD was assembled onto a rough Au-electrode[8] by immobilization of a primary cystamine monolayer followed by coupling of glutaric dialdehyde and subsequent covalent linkage of SP-GOD to the functionalized monolayer.

Fig. 1 shows the cyclic voltammograms obtained upon bioelectrocatalysed oxidation of glucose in the presence of ferrocene carboxylic acid, as diffusional electron mediator, using the photoisomerizable enzyme electrode. The electrode was illuminated, $\lambda > 495$ nm, to generate the SP-GOD monolayer. A high electrocatalytic anodic current is observed [curve (a)] indicating the effective bioelectrocatalysed oxidation of glucose. Photoisomerization of the electrode, $320 < \lambda < 360$ nm, yields the MRH^+-GOD monolayer. This is accompanied by a decrease in the electrocatalytic anodic current [curve (b)]. Further regeneration of the SP-GOD monolayer by irradiation of the MRH^+-monolayer, $\lambda > 495$ nm, regenerates the high amperometric response, and reisomerization of the SP-GOD monolayer to the MRH^+-GOD electrode again retards the electrocatalytic oxidation of glucose. Thus, dinitrospiropyran-modified GOD exhibits reversible 'ON–OFF' photochemically-induced bioelectrocatalytic properties. The SP-GOD represents a 'switched-on' biocatalyst, where MRH^+-GOD is a partially switched-off enzyme.

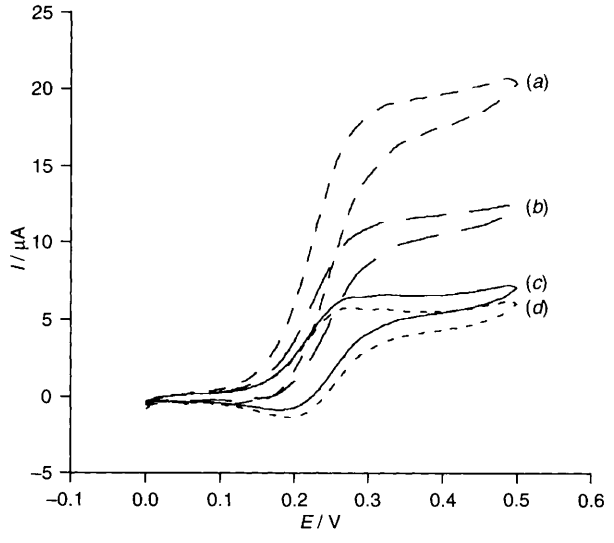

Fig. 1 Cyclic voltammograms obtained upon bioelectrocatalysed oxidation of glucose (50 mmol dm^{-3}) in the presence of ferrocene carboxylic acid (4 × 10^{-4} mol dm^{-3}) as diffusional electron mediator: (*a*) with the SP-GOD monolayer electrode and the absence of DNP-Ab; (*b*) with the MRH^+-GOD monolayer electrode in the presence or absence of DNP-Ab; (*c*) with the SP-GOD monolayer electrode in the presence of DNP-Ab, 20 mg ml^{-1}. Curve (*d*) shows the cyclic voltammogram with either the MRH^+-GOD or SP-GOD monolayer electrodes in the presence of ferrocene carboxylic acid, 4 × 10^{-4} mol dm^{-3}, and in the absence of glucose. All experiments were recorded in potassium phosphate (0.1 mol dm^{-3}, pH = 7.0) under argon, potential scan rate, 2 mV s^{-1}.

The cyclic voltammograms obtained upon bioelectrocatalysed oxidation of glucose by the photoisomerizable enzyme-electrode, in the presence of the anti-dinitrophenyl-antibody, DNP-Ab are also shown in Fig. 1. The experiment is initiated by the application of the SP-GOD monolayer electrode in the presence of ferrocene carboxylic acid as diffusional electron mediator and glucose. A high amperometric response [curve (*a*)] is obtained, indicating the effective bioelectrocatalysed oxidation of glucose. Addition of the DNP-Ab results in a substantial decrease in the electrocatalytic current [curve (*c*)] and only the background current is detected.

The blocking of the electrocatalytic oxidation of glucose by the SP-GOD monolayer electrode in the presence of DNP-Ab is attributed to the antigenic properties of the dinitrospiropyran units for DNP-Ab.[9] Association of the DNP-Ab to the dinitrospiropyran units prevents the mediated electron transfer to GOD, and consequently, the electrobiocatalysed oxidation of glucose is blocked. Further irradiation of the SP-GOD monolayer-electrode in the presence of DNP-Ab, $320 < \lambda < 360$ nm yields the MRH$^+$-monolayer electrode. The dinitromerocyanine units lack antigen properties for DNP-Ab.[9] Dissociation of the DNP-Ab from the enzyme monolayer reactivates the partial bioelectrocatalysed oxidation of glucose by the MRH$^+$-GOD monolayer electrode [curve (*b*)]. It should be noted that while SP-GOD electrode in the absence of DNP-Ab represented a fully switched-on state, the same electrode in the presence of DNP-Ab is entirely switched-off and the MRH$^+$-GOD monolayer with DNP-Ab is the switched-on state in the system. Further irradiation of the MRH$^+$-GOD electrode, $\lambda > 495$ nm, with the DNP-Ab, regenerates the SP-GOD monolayer and the electrocatalytic oxidation of glucose is blocked [curve (*c*)]. By further isomerization of the monolayer to the MRH$^+$-GOD the electrobiocatalyst is reactivated [curve (*b*)]. Under these conditions, DNP-Ab is not associated with the enzyme. By elimination of the DNP-Ab electrolyte solution, followed by the introduction of an Ab-free ferrocene carboxylic acid and glucose solution, the original system is reassembled. Photoisomerization of the MRH$^+$-GOD monolayer electrode to the SP-GOD state regenerates the original amperometric response of the fully switched-on enzyme [curve (*a*)] indicating the effective bioelectrocatalysed oxidation of glucose.

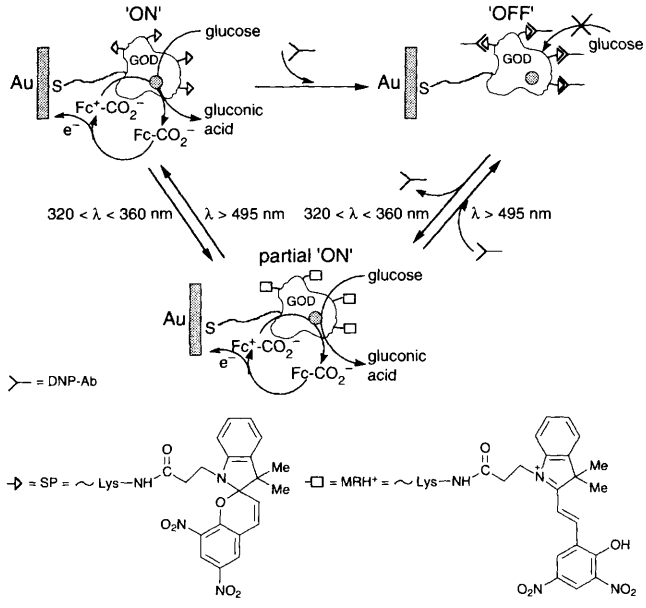

Fig. 2 Schematic operation of the biphasic switch of a dinitrospiropyran-GOD monolayer electrode in the presence or absence of the DNP-Ab

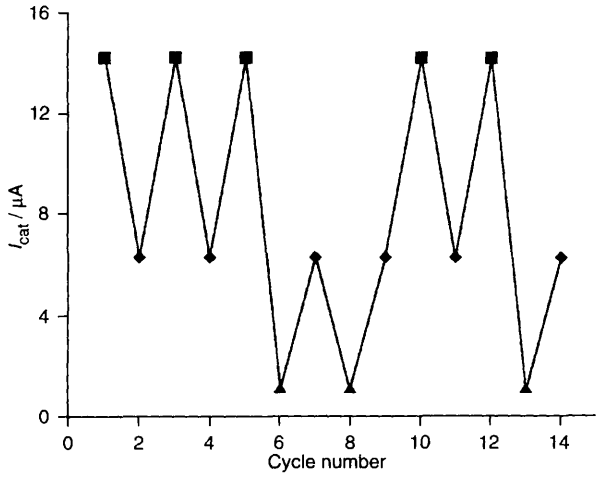

Fig. 3 Cyclic amperometric tranduction of the biphasic switch composed of the dinitrospiropyran-GOD monolayer electrode in the absence and presence of DNP-Ab. (■) SP-GOD monolayer electrode without DNP-Ab. (◆) MRH$^+$-GOD monolayer electrode with or without DNP-Ab. (▲) SP-GOD monolayer electrode in the presence of DNP-Ab.

Thus, dinitrospiropyran-modified glucose oxidase and DNP-Ab system exhibits a biphasic switch operation for the amperometric transduction of recorded optical signals, Fig. 2. The SP-GOD/MRH$^+$-GOD-electrode represents one partial photochemical switch where the SP-GOD and DNP-Ab and MRH$^+$-GOD and DNP-Ab-electrode is the second photochemical switch. The two photoswitches are coupled and elimination or introduction of the DNP-Ab transforms one photoswitch into the other. The sequence of cyclic activation and deactivation of the amperometric responses by the two photochemical switches is summarized in Fig. 3. The operation of the biphasic optobioelectronic switch can be described by an analogous electronic-circuit that includes two coupled 'OR' and 'OR' switches.

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