Magneto-Switchable Bioelectrocatalysis

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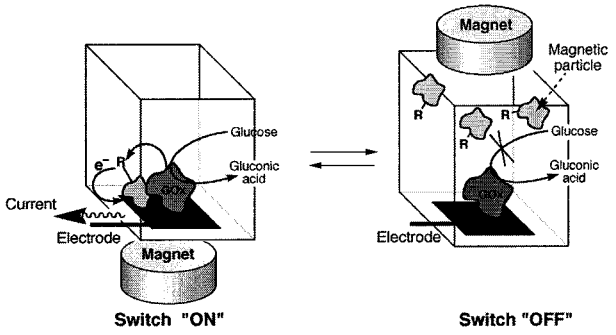
Bioelectronics[1] is a rapidly developing scientific field aimed to integrate biomaterials, e.g., enzymes, antigens/antibodies, or DNA with electronic transducers. Electrically contacted enzyme electrodes or protein-functionalized electrodes are employed as biosensors,[2] electrocatalytic interfaces for biofuel cells,[3] or biomaterial-based electronic elements.[4] Reversible activation and deactivation of biomaterial functions by external triggering signals and, specifically, the photochemical "ON-OFF" switching of biological functions provides the basis for optobioelectronics.[5] The amperometric transduction of optical signals recorded by photoswitchable redox-enzyme assemblies was suggested as information storage systems and light signals amplifiers.[6−9] Other potential applications of switchable biomaterials include the development of targeted therapeutic materials,[10] the tailoring of reversible immunosensors[11] or DNA sensors,[12] and the design of novel matrices for affinity chromatography.

The present report addresses for the first time the magnetic control of bioelectrocatalysis, and paves the new concept of magneto-bioelectronics. Scheme 1 outlines the method to control

Scheme 1. Magneto-Switched Bioelectrocatalyzed Oxidation of Glucose in the Presence of Relay-Functionalized Magnetic Particles



by an external magnetic field the bioelectrocatalytic oxidative functions of a redox enzyme, e.g., glucose oxidase. Magnetic particles functionalized with a relay unit, R, are used to electrically contact the redox enzyme and the electrode. Positioning of a magnet below the electrode attracts the magnetic particles to the electrode. This enables the oxidation of the relay unit by the conductive support, and subsequently activates the bioelectrocatalyzed oxidation of the substrate by the oxidized relay associated with the magnetic particles. Transfer of the external magnet to an upper position lifts the magnetic particles upward, and removes them from the electrode support. This prevents the oxidation of the relay units and the bioelectrocatalyzed oxidation of the substrate is switched-off. By alternate positioning of the external magnet below and above the electrochemical cell, the bioelectrocatalyzed oxidation of the substrate is reversibly switched between "ON" and "OFF" states, respectively. The magnetic control of a bioelectrocatalytic reduction process can be similarly controlled by the use of relay-functionalized magnetic particles that are reduced at the electrode support.

Magnetite particles[13] (Fe_3O_4), ca. 1 μm average diameter, were silylated with [3-(2-aminoethyl)aminopropyl]trimethoxysilane and *N*-(ferrocenylmethyl)aminohexanoic acid[9b] (**1**), or *N*-methyl-*N*′-(dodecanoic acid)-4,4′-bipyridinium,[14] (**2**), redox functions were covalently linked to the particle surface using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as a coupling reagent, Scheme 2.

Scheme 2. Synthesis of Relay-Functionalized Magnetite Particles

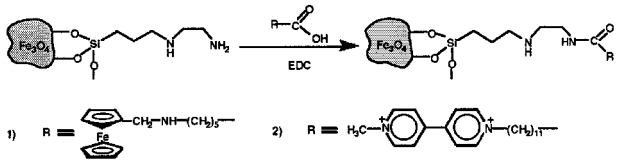


Figure 1A shows the magnetic-field control of the electrochemistry of the ferrocene-functionalized magnetite particles using differential pulse voltammetry. Positioning of the external magnet below the electrode attracts the magnetic particles and stimulates the oxidation of the ferrocene units, $E^\circ = 0.31$ V vs SCE. Transfer of the magnet to the upward position blocks the electrochemistry of the ferrocene units. By cyclic positioning of the external magnet below and above the cell, the electrical response of the ferrocene sites is reversibly switched between "ON" and "OFF" configurations, respectively (Figure 1A, inset). By coulometric analysis of the cathodic (or anodic) wave in the cyclic voltammogram of the ferrocene-functionalized magnetic particles, and knowing the size of the particles, we estimate the surface coverage of ca. 1000−3000 ferrocene units per particle.

Figure 1B shows the magnetic control of the bioelectrocatalyzed oxidation of glucose, 80 mM, using glucose oxidase, GOx, and the ferrocene-functionalized particles. Magnetic attraction of the particles to the electrode results in the oxidation of the ferrocene units and the mediated activation of GOx. The bioelectrocatalyzed oxidation of glucose is evident by the resulting electrocatalytic anodic current. Positioning of the magnet above the cell removes the magnetic particles from the electrode, and this switches off the bioelectrocatalyzed oxidation of glucose. By the cyclic placement of the magnet below and above the electrode support, the bioelectrocatalyzed oxidation of glucose is reversibly switched

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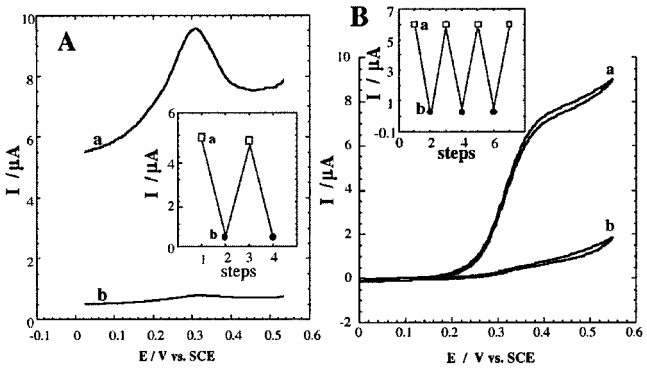

Figure 1. (A) Differential pulse voltammetry (DPV) of ferrocene-functionalized magnetite particles: (a) magnet below the cell and (b) magnet above the cell. Inset: Reversible magneto-switched redox responses (peak current of DPV) of ferrocene-modified magnetite. Data were recorded at a potential scan rate of 20 mV s^{-1}, in the presence of the Au electrode (0.8 cm^2) and ferrocene-functionalized magnetite particles (10 mg) in 0.1 M phosphate buffer (pH 7.0, 2 mL) under Ar. (B) Cyclic voltammograms corresponding to the magneto-switched bioelectrocatalyzed oxidation of glucose by glucose oxidase, GOx: (a) magnet below the cell and (b) magnet above the cell. Inset: Reversible magneto-switched amperometric transduction of the bioelectrocatalyzed oxidation of glucose. Data were recorded at a potential scan rate of 5 mV s^{-1}, in the presence of the Au-electrode (0.8 cm^2), ferrocene-functionalized magnetite particles (10 mg), glucose (80 mM), and GOx (1 mg mL^{-1}) in 0.1 M phosphate buffer (pH 7.0, 2 mL) under Ar.

between "ON" and "OFF" states (Figure 1B, inset). Control experiments reveal that nonfunctionalized magnetic particles do not activate the enzyme for the bioelectrocatalyzed oxidation of glucose, implying that the ferrocene units mediate the electrical activation of GOx. Also, the use of an external magnet for the bioelectrocatalyzed activation of GOx is important, and the physical precipitation of the particles on the electrode by settling is very slow (several hours). Similarly, confinement of the ferrocene-functionalized particles in the upper position for several hours, by the application of the external magnetic field, does not lead to the evolution of any bioelectrocatalytic current. This implies that the redox relay tethered to the magnetic particle is not dissociated from the heterogeneous carrier during the time scale of the experiments.

The magnetic switching of the reductive bioelectrocatalytic functions of an enzyme was demonstrated by the use of the bipyridinium-functionalized magnetite particles that activate the bioelectrocatalyzed reduction of nitrate (NO_3^-) to nitrite (NO_2^-) in the presence of nitrate reductase, NR (EC 1.6.6.2).

Figure 2A shows the differential pulse voltammogram upon the magnetic control of the electrochemical properties of the bipyridinium redox units. The magnetic attraction of the magnetic particles to the electrode results in the reduction of the bipyridinium units, $E^\circ = -0.55$ V vs SCE. The loading of the magnetic particles with the bipyridinium redox units has been found to be ca. 800−2000 units per particle. Figure 2B shows the magnetic control of the bioelectrocatalyzed reduction of NO_3^- to NO_2^- by NR. Positioning of the magnet below the electrode stimulates the electrochemical reduction of the bipyridinium salt to the respective radical cation that acts as an electron mediator for NR.[15] The

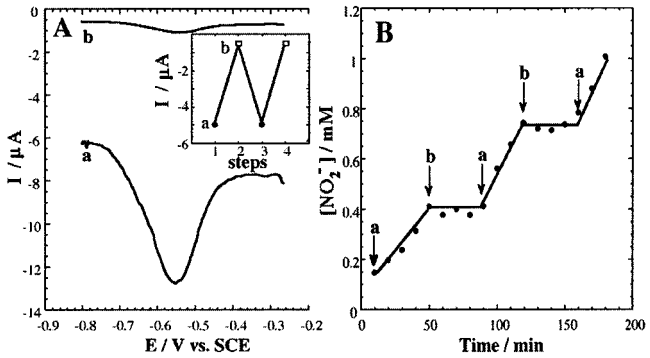

Figure 2. (A) Differential pulse voltammetry (DPV) of bipyridinium-functionalized magnetite particles: (a) magnet below the cell and (b) magnet above the cell. Inset: Reversible magneto-switched redox responses (peak current of DPV) of bipyridinium-modified magnetite. Data were recorded at a potential scan rate of 20 mV s^{-1}, in the presence of the Au-electrode (0.8 cm^2), bipyridinium-functionalized magnetite particles (10 mg) in 0.1 M phosphate buffer (pH 7.0, 2 mL) under Ar. (B) Magnetic-field control of the rate of the nitrate reductase, NR, bioelectrocatalyzed reduction of NO_3^- to NO_2^- under constant potential electrolysis: (a) magnet below the cell and (b) magnet above the cell. Data were recorded at $E = -0.7$ V vs SCE in the presence of the Au-electrode (0.8 cm^2), bipyridinium-functionalized magnetite particles (10 mg), KNO_3 (1 mM), and NR (5 units per mL) in 0.1 M phosphate buffer (pH 7.0, 2 mL) under Ar.

bioelectrocatalyzed reduction of NO_3^- proceeds as long as the particles are attracted to the electrode by the magnet, and a negative potential −0.7 V (vs SCE) is applied on the electrode. Positioning of the magnet above the electrode removes the magnetic particles from the electrode, and the biocatalyzed reduction of NO_3^- is blocked even upon the application of a negative potential in the electrode. Further attraction of the particles to the electrode by the external magnetic field reactivates the bioelectrocatalytic functions of the enzyme (Figure 2B, inset). Control experiments reveal that nonmodified magnetite particles do not activate the bioelectrocatalytic properties of NR. These results imply that the electrogenerated magnetite-linked bipyridinium radical-cation indeed mediates electron transfer to nitrate reductase.

In conclusion, the present study has demonstrated for the first time magneto-switchable bioelectrocatalysis. One may envisage various future applications of magneto-controlled redox enzymes in the external control of catalytic transformations in bioreactors, the tailoring of reversible amperometric immunosensors, the regeneration of enzyme-biosensor electrodes, and the external triggering of biofuel cells. Specifically, the external magnetic organization of the functionalized particles on surfaces, and the subsequent activation of enzymes, may be employed to pattern surfaces by external magnetic masks. We believe that the present study opens a new topic of magnetobioelectronics.

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