Bioelectrocatalysis

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Adaptive Orientation of Multifunctional Nanowires for Magnetic Control of Bioelectrocatalytic Processes**

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Bioelectronics, the coupling of biomaterials and electronic devices, is a major interdisciplinary research area.[1,2] In one rapidly growing area of bioelectronics, magneto-bioelectronics, external magnetic fields are used to control bioelectrochemical processes.[2] Hirsch et al.[3] were the first to report on magnetoswitchable bioelectrocatalysis in connection to relay-modified magnetic spheres. Recent developments in the use of functionalized microparticles for switching bioelectrocatalytic reactions (in the presence of soluble enzymes) have been reviewed.[2] New adaptive nanomaterials capable of providing semi-analogue control of bioelectrochemical processes (in addition to "on/off" switching) should greatly enhance the power and capabilities of magneto-bioelectronics.

Here we wish to report on the use of enzyme-functionalized nanowires for the magnetic control of bioelectrocatalytic transformations, without removal of the biocatalyst from the surface. Nanowires have recently received considerable attention as potential components for functional nanoelectronic devices.[4] We describe here how nanowires can add a unique dimension to magneto-bioelectronics, as they facilitate reversible modulation and fine-tuning of bioelectrocatalytic processes, in addition to the "on/off" switching common to functionalized magnetic spheres.[2,3] Recently we demonstrated that catalytic nickel nanowires can be used for magnetic control of the electrochemical reaction of methanol through reversible changes in the nanowire orientation.[5] In the present work we used two-segment gold/nickel nanowires—prepared by a template-guided synthesis and functionalized with glucose oxidase (GOx)—for controlling the biocatalytic oxidation of glucose, in connection to a surface-bound ferrocene (Fc) electron-transfer mediator. Spatially selective functionalization with the enzyme was accomplished by application of a self-assembled monolayer (SAM) of mercaptoacetic acid (MAA) onto the gold segment, followed by electrostatic attraction of the polyethyleneimine (PEI) polycation and of the negatively charged enzyme. Figure 1 depicts the new setup for nanowire-based magnetoswitchable tuning of bioelectrocatalytic processes. Positioning the functionalized nanowire in the horizontal orientation ensures effective contact of the enzyme and the surface-bound mediator, and leads to a mediated activation of GOx (Figure 1 A). Switching the nanowires to the vertical position greatly hinders the communication between the nanowire-confined GOx and the surface Fc relay (Figure 1 B), while retracting the nanowires from the surface (by moving the magnetic field) totally blocks the mediated activation of GOx (Figure 1 C). Such magnetically modulated bioelectrocatalytic transformations can be repeated multiple times when the surface orientation of the GOx-functionalized nanowires is switched between the vertical and horizontal positions. To the best of our knowledge, this is the first example illustrating the reorientation of a surface-confined enzyme for tuning bioelectrocatalytic transformations. This adaptive orientation of biocatalytic nanowires holds great promise for the external control of devices ranging from biosensors to biofuel cells, in response to specific needs. Multisegment nanowires offer the

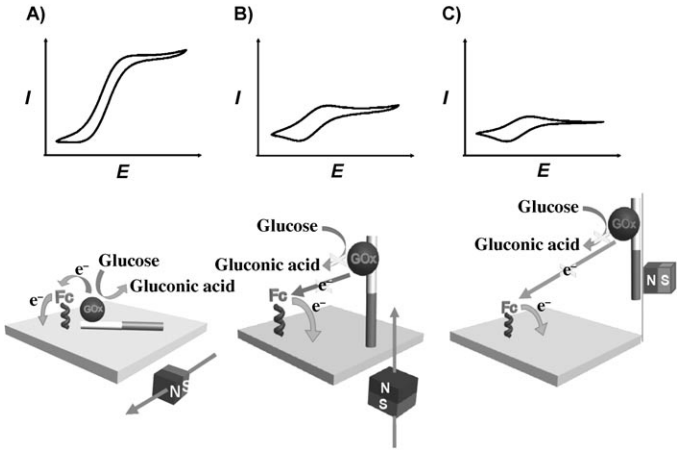

Figure 1. Nanowire-based magnetoswitchable bioelectrocatalytic processes. In the experimental setup with the GOx–gold/nickel nanowires and the Fc-modified surface, the magnetic field can be oriented in the horizontal (A), vertical (B), and "off" (C) positions, for activating (A), hindering (B), and blocking (C) the communication between the nanowire-confined GOx and the surface Fc relay. The corresponding cyclic voltammograms are also shown.

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multifunctionality necessary for such operations: the nickel segment provides the magnetic control, while the gold part supports the biocatalytic activity.

Figure 2 illustrates the magnetic tuning of the bioelectrocatalyzed oxidation of glucose using the GOx–gold/nickel nanowires and the Fc-modified electrode support. It shows

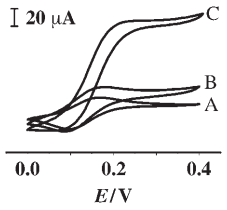

Figure 2. Cyclic voltammograms for 20 mM glucose with the GOx–Au/Ni nanowires in the "off" (A), vertical (B), and horizontal (C) positions. Potential scan from 0.0 to 0.4 V (vs. Ag/AgCl) at 10 mVs^{-1}; electrolyte: 0.1 M phosphate buffer (pH 7.0).

the cyclic voltammetric response for glucose (over the range from 0.0 to +0.4 V) in the absence of the nanowires, and with the nanowires magnetically oriented in the vertical and horizontal positions (Figure 2A–C). As expected, a small redox signal characteristic of a surface-confined Fc mediator ($E^{\circ} \approx 0.15$ V) is observed in the absence of the magnetic field (that is, the enzyme-functionalized nanowires; Figure 2A). Positioning the nanowires on the surface with the magnetic field in the horizontal orientation leads to a mediated activation of GOx and to a large sigmoidal anodic response (Figure 2C) characteristic of bioelectrocatalytic processes. Reorienting the nanowires from the horizontal to the vertical position (Figure 2B) blocks most of the bioelectrocatalytic oxidation and leads to a substantially smaller current signal. A number of factors affect the relative signals in the vertical and horizontal orientations (see below).

The reversible modulation of the mediated activation of GOx upon repeated changes of the surface orientation is illustrated by the current–time recording obtained during such cyclic reorientation of the GOx–gold/nickel nanowires (Figure 3C). Reversible changes in the current output are observed upon switching the surface orientation of the nanowires between the vertical and horizontal positions. Such magnetoswitchable bioelectrocatalytic current changes are highly reproducible, with relative standard deviations of 3.3 and 3.7% for the vertical and horizontal positions, respectively. Control experiments reveal that the unmodified surface (without the Fc) does not activate the enzyme (Figure 3B). Similarly, no bioelectrocatalytic oxidation is observed with the nonfunctionalized nanowires, in other words, in the absence of GOx (Figure 3A). Apparently (and as expected), both the enzyme and mediator are essential for the mediated activation of GOx. As evident in Figure 4, increasing the substrate concentration (in 5-mM steps) results in well-defined cyclic voltammograms, with larger bioelectrocatalytic currents for higher levels of glucose. The bioelectrocatalytic current is proportional to the glucose concentration.

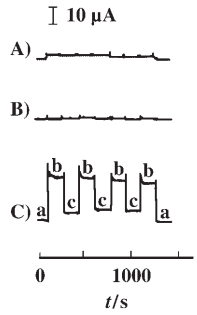

Figure 3. Magnetically modulated mediated activation of GOx. Amperometric response for 5 mM glucose A) with the Fc-modified Au electrode but without the GOx–Au/Ni nanowires, B) with GOx–Au/Ni nanowires but without the Fc modification, and C) with both the Fc-modified Au electrode and the GOx–Au/Ni nanowires. Nanowires in the "off" (a), vertical (b), and horizontal (c) positions. Working potential: +0.2 V (vs. Ag/AgCl); electrolyte: 0.1 M phosphate buffer (pH 7.0).

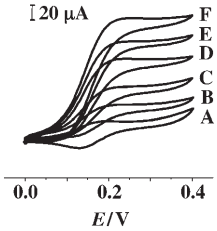

Figure 4. Cyclic voltammograms for the magnetically triggered bioelectrocatalytic reaction with increasing glucose concentrations (5–25 mM; B–F), along with the background voltammogram (A). GOx–Au/Ni nanowires are oriented in the horizontal position. Conditions, as in Figure 2.

Based on Figure 1, one would expect that the vertical orientation of the GOx–gold/nickel nanowires would lead to a negligible bioelectrocatalytic response. The slightly larger response in the vertical position is not surprising considering that both ends of the nanowires, the nickel and modified gold ones, touch the electrode surface. This behavior reflects the random polarization of the nickel segment, which is characteristic of nickel segments and rods with large aspect ratios. These segments act as single-domain nanomagnets with a longitudinal magnetization.[6] The magnetic polarization of such longitudinal nanomagnets during the template-guided growth is mixed randomly such that the whole array (of nanomagnets within the membrane pores) is collectively demagnetized.[7] As evident in Figure 5, this results in a random polarization of the nickel nanomagnets relative to the modified gold segment. Hence, both the nickel and modified-

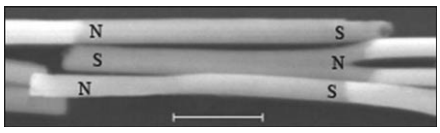

Figure 5. SEM image of grouped gold/nickel nanowires illustrating the relative polarities of the nickel segment (bright segment is gold). Scale bar: 1 μm.

gold ends of the nanowires touch the electrode surface when they are placed in the vertical orientation. Under these conditions, the GOx confined to the bottom of the wires can contact the Fc surface relay. The amount of the enzyme at the end of the nanowires (that is, the contacting mediator) is substantially less than that confined on the walls of the gold segment. The relative amounts of GOx at the bottom and wall of the nanowires depend upon their aspect ratio and the length of the gold segment. This allows fine-tuning of the bioelectrocatalytic reaction using nanowires of different dimensions. For example, a larger cyclic-voltammetric anodic response (ranging from 7.5 to 22.5 μA) was observed for 20 mM glucose when the length of gold segment was increased (using plating charges between 5 to 20 C, respectively), in other words, for higher GOx loading per nanowire (not shown). Yet, independent of the wire dimensions, the amount of GOx in contact with the surface in the vertical orientation is substantially less than that in the horizontal orientation. No enzyme functionalization of the nickel surface is expected in the absence of the MAA self-assembly under the experimental conditions of this study.[8] The template fabrication route allows one to readily control the relative length of the gold and nickel segments and hence to fine-tune the modulation of the bioelectocatalytic response.

In conclusion, the present study has demonstrated for the first time the use of adaptive functional nanowires for magnetoswitchable bioelectrocatalysis. It also represents the first example of tuning bioelectrocatalytic transformations through reorientation of a surface-confined enzyme without removing the biocatalyst from the surface. This is in contrast to the "on/off" switching possible by attraction and retraction of functionalized magnetic spheres.[2,3] While the concept has been presented in connection with the mediated activation of GOx, it can be readily extended to a wide range of biocatalytic systems. Such nanowire-based magnetic tuning of enzymatic reactions holds great promise for regulating the operation of bioreactors, biofuel cells, and biosensing devices in response to specific needs.

Experimental Section

Cyclic voltammograms were recorded by the μAutolab Potentiostat type II (Eco Chemie BV, Utrecht, Netherlands). The electrode assembly consisted of a microscope glass slide (7.5 cm × 2.5 cm × 0.1 cm), which was coated with a 0.06-mm-thick gold layer (99.99% pure), that served as working electrode, and a glass tube 2 cm in height and 1.25 cm in diameter which was capped at the end affixed to the slide. The glass tube was glued to the center of the glass slide to define the electrochemical cell. Electrical contact between the gold electrode and a copper wire was achieved using Ag/AgCl ink (Ercon, Wareham, MA, USA). The reference and counter electrodes were Ag/AgCl (Model CHI111, CH Instruments, Austin, TX, USA) and a 0.5-mm-diameter platinum wire, respectively. The magnetic orientation of the functionalized gold/nickel nanowires on the electrode surface was possible by selectively positioning an external cube-shape NdFeB/Ni-coated magnet (1.1 cm × 1.1 cm × 1.1 cm, 12.4 kG) under the glass slide. The Au/Ni nanowires were characterized by scanning electron microscopy (SEM) using an XL30 SEM instrument (FEI Co., Hillsboro, OR, USA).

All aqueous solutions were prepared from 18 MΩ nanopure water (purified using an ELGA Purelab Ultra (model ULTRA SCIENCETIFIC)). The majority of chemicals were purchased from Sigma-Aldrich, while the gold-plating solution was obtained from Technic (Cranston, RI, USA) and hydrochloric acid was obtained from EMD Chemicals (Merck, Germany). Anodisc alumina membranes, with a pore size of 200 nm and thickness of 60 μm, were purchased from Whatman (catalogue no. 6809-6022; Maidstone, UK).

Gold–nickel nanowires (≈20 μm long and ≈200 nm diameter) were prepared by electrochemical deposition into the nanopores of the alumina membrane template, by controlling the electrodeposition charge in accordance to a previous procedure.[9] A silver film was first sputtered on one side of the template to serve as a working electrode. The membrane was then assembled in a plating cell where aluminum foil was used as a contact for the sputtered silver. Initially, 10 coulombs (C) of copper was electrodeposited from a 1M $CuSO_4 \cdot 5H_2O$ solution at −1.0 V (vs. Ag/AgCl) in connection to a Pt wire counter electrode; subsequently, 20 C of gold was plated at −0.9 V from the commercial gold-plating solution; finally, 20 C of nickel was electrodeposited at −1.0 V from a solution containing 20 g L^{-1} $NiCl_2 \cdot 6H_2O$, 515 g L^{-1} $Ni(H_2NSO_3)_2 \cdot 4H_2O$, and 20 g L^{-1} H_3BO_3 (buffered to pH 3.4). Following the nickel electrodeposition, the copper and silver sputtered layers were dissolved in a 0.5M CuCl solution (in 30% HCl).[9] The nanowires were removed from the template by dissolving the alumina in an agitated 3M NaOH solution for 30 min. The resulting Au/Ni nanowires were repeatedly washed with water to remove residual base and salts. After the washing step, these nanowires were collected by placing a small magnet on the side of the flask and were suspended in a phosphate buffer (PB) solution (0.1M, pH 7.0) for storage.

The surface of the gold segment of the Au/Ni nanowires was modified by self-assembly of mercaptoacetic acid (MAA), followed by electrostatic adsorption of the polycationic PEI and of the anionic GOx. This coating procedure was based on the protocol outlined by Rodriguez and Rivas,[10] and was carried out by immersing the Au/Ni nanowires in 500 μL of 0.2M MAA in ethanol overnight. The MAA-modified nanowires were then collected with a magnet, and the supernatant was removed. The wires were then washed twice with absolute ethanol and twice with water. Subsequently, 400 μL of 10 mg mL^{-1} PEI in PB was added to the wires, and the PEI layer was allowed to form for 30 min. After two washing steps with PB, the enzyme was immobilized by adding 400 μL of PB solution containing 2 mg mL^{-1} GOx. The coated wires were washed again twice and stored in 400 μL of PB.

Gold working electrodes functionalized with a ferroceneacetic acid monolayer were prepared based on a modification of the protocol of Katz et al.[11] The gold electrode was placed in 10 mM cysteamine aqueous solution for 2 h to yield an amino-functionalized surface. This amino-functionalized gold electrode was exposed to a 5 mM ferroceneacetic acid/HEPES buffer (0.1M, pH 7.2) solution containing 1 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) for 3 h.

Measurements were performed in a PB supporting electrolyte medium. Cyclic voltammetric measurements were carried out under quiescent conditions, with the magnet in the horizontal, vertical, or "off" positions. The mediated bioelectrochemical oxidation was triggered by positioning the magnet below the corresponding working electrode for attracting the GOx–Au/Ni nanowires to the surface, and recording the cyclic voltammograms (between 0.0 and + 0.4 V vs. Ag/AgCl at 10 mV s^{-1}) or the corresponding amperogram (at + 0.2 V vs. Ag/AgCl). The reorientation of the GOx–Au/Ni nanowires from the horizontal to the vertical surface positions was accomplished by rotating the magnet 90° under the bottom of the cell. The "off" position corresponded to the external magnet attracting the Au/Ni nanowires to the upper side wall of the cell, away from the working

electrode area. All measurements were performed at ambient temperature (ca. 24 °C).

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