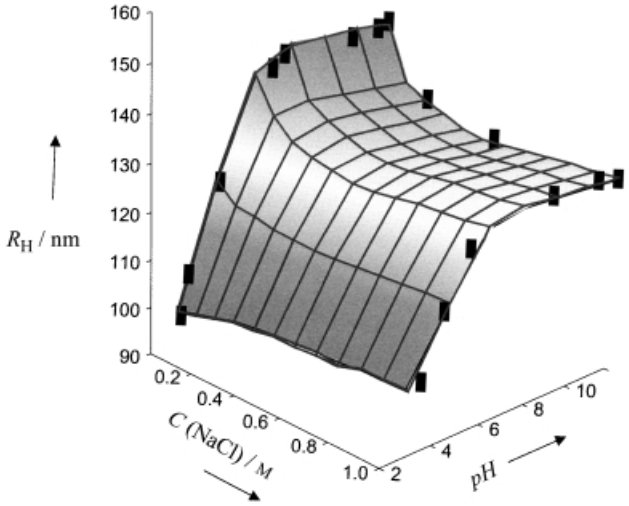

Figure 6. Hydrodynamic radius (R_H) of PB_{40}-*b*-PGA_{100} vesicles measured by DLS studies as a function of ionic strength (NaCl salt concentration) and pH value. ■: experimental data.

by using the 1,2-vinyl bonds present in the polybutadiene. First experiments have shown a moderate shrinkage of the vesicle size after UV curing, which is a typical phenomenon accompanying the cross-linking of polybutadiene molecules. Such nanoparticles might be suitable for a number of applications including the encapsulation and/or the release of hydrophilic as well as hydrophobic active species or their use as sensor nanodevices.

Experimental Section

PB_{40}-*b*-PGA_{100}: Anionic polymerization of butadiene in THF at $-78\,^{\circ}C$ using sec-butyllithium as initiator followed by quenching the oligobutadienyllithium with 1-(3-chloropropyl)-2,2,5,5-tetramethyl-1-aza-2,5-disilacyclopentane and acidic aqueous workup gave the ω-aminooligobutadiene. The oligomer functionalized at the primary amine end was then used to initiate the ring-opening oligomerization of Bn-GluNCA in DMF.[16] The length of the γ-benzyl-L-glutamate segment could be controlled through the molar ratio of Bn-GluNCA to ω-aminooligobutadiene initiator to give the required polybutadiene-*b*-poly(γ-benzyl-L-glutamate) block copolymer. After removal of the benzyl ester groups by hydrogenation, the amphiphilic diblock copolymer PB_{40}-*b*-PGA_{100} was obtained. The composition of the block copolymer was analyzed by means of ^1H and ^{13}C NMR spectroscopy and gel permeation chromatography.

SLS and DLS measurements were performed on a ALV5000 goniometer equipped with a ALV5000/E Multiple Tau digital Realtime correlator. The R_H and PDI values of the aggregates were obtained by a cumulant and CONTIN analysis of the experimental correlation functions. TEM pictures were recorded on a JEOL JEM100S microscope working at 80 KV. Samples were prepared by freeze–fracture process on 1.25 g L^{-1} copolymer solutions (7/3 water/glycerol). Fluorescence spectra were recorded on a SAFAS Spectrofluorometer flx spectrometer. CD experiments were carried out on a JOBIN YVON CD6 Spex spectrometer (184–900 nm).

Received: December 21, 2001 [Z18426]

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Dual Biosensing by Magneto-Controlled Bioelectrocatalysis**

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In the last two decades extensive research efforts were directed towards the development of amperometric biosensors based on redox-active enzymes.[1, 2] Ingenious methods to electrically contact redox enzymes with electrode supports by tethering relay units to the protein,[3] the immobilization of

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[**] This research is supported by the German–Israel Project Cooperation (DIP). I.W. acknowledges the Max Planck Award for International Cooperation.

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  1433-7851/02/4108-1343 $ 20.00+.50/0

the biocatalysts in redox polymers,[4] or the reconstitution of the enzymes with relay–cofactor units,[5] were developed. The development of multifunctional electrodes that allow the simultaneous analysis of two (or more) substrates remains, however, a challenging subject in bioelectronics. The use of a photoswitchable enzyme as a biocatalytic component in a two-enzyme bifunctional sensor system was suggested as a means to analyze two different substrates by an integrated biosensor.[6] That is, in the photochemically switched "off" state of the enzyme, the amperometric response corresponding to the analysis of one of the substrates is recorded, whereas in the photochemically switched "on" state of the second enzyme the cumulative amperometric response, which corresponds to the analysis of the sum of the substrates is recorded. Thus, by a two-step detection procedure, the two substrates can be analyzed quantitatively. Besides the complexity of this concept, the phototriggered redox enzymes cannot be fully switched off and on, and therefore a different methodology to resolve this basic goal is required. Recently we reported the magnetic control of bioelectrocatalyzed transformations by the application of redox-relay-functionalized magnetite particles, and an external magnetic field as a triggering signal.[7] In contrast to extensive research efforts that utilize magnetic particles for the separation or concentration of molecular or biomolecular components,[8, 9] our approach of tethering redox-active units to magnetic particles, and the accompanying magnetically controlled electrochemical activation of chemical transformations, is a novel concept in electrocatalysis,[10] and bioelectrocatalysis.[7] Here we report the dual analysis of two substrates by the application of two enzymes, a relay-monolayer-functionalized electrode, relay-NAD^+-cofactor-functionalized magnetic particles, and the use of an external magnetic field.

Pyrroloquinoline quinone (PQQ; **1**), acts as an electrocatalyst for the oxidation of reduced 1,4-dihydronicotinamide adenine dinucleotide (NADH).[11] Accordingly, aminopropylsiloxane-functionalized magnetic particles were modified with **1**. Aminoethyl-functionalized-NAD^+ ($aNAD^+$; **2**)[12] was coupled to the PQQ-modified magnetic particles. By coulometric analysis of the redox-wave of PQQ in the cyclic voltammogram of the $aNAD^+$-PQQ-functionalized magnetic particles, and knowing the size of the particles, we estimated the surface coverage to be around 1000–3000 PQQ units per particle. Earlier we demonstrated that the PQQ/$aNAD^+$ ratio in the assembly is approximately 1.[13] A cystamine-functionalized Au-electrode was modified with *N*-(ferrocenylmethyl)aminohexanoic acid (Fc; **3**).[14] Coulometric analysis of the redox wave of the Fc units indicates a surface coverage corresponding to about 5×10^{-11} mol cm^{-2}.

The analysis system (Figure 1) consists of the Fc-modified Au-surface as a working electrode, the $aNAD^+$-PQQ-functionalized magnetic particles, the two enzymes, glucose oxidase (GOx; E.C.1.1.3.4 from *Aspergillus niger*), and lactate dehydrogenase (LDH; E.C.1.1.1.27 from rabbit muscle, type II), and the two substrates, glucose and lactic acid. Figure 2A shows the cyclic voltammogram observed for the system that lacks the two substrates, with the external magnet positioned above the electrochemical cell. Only the redox

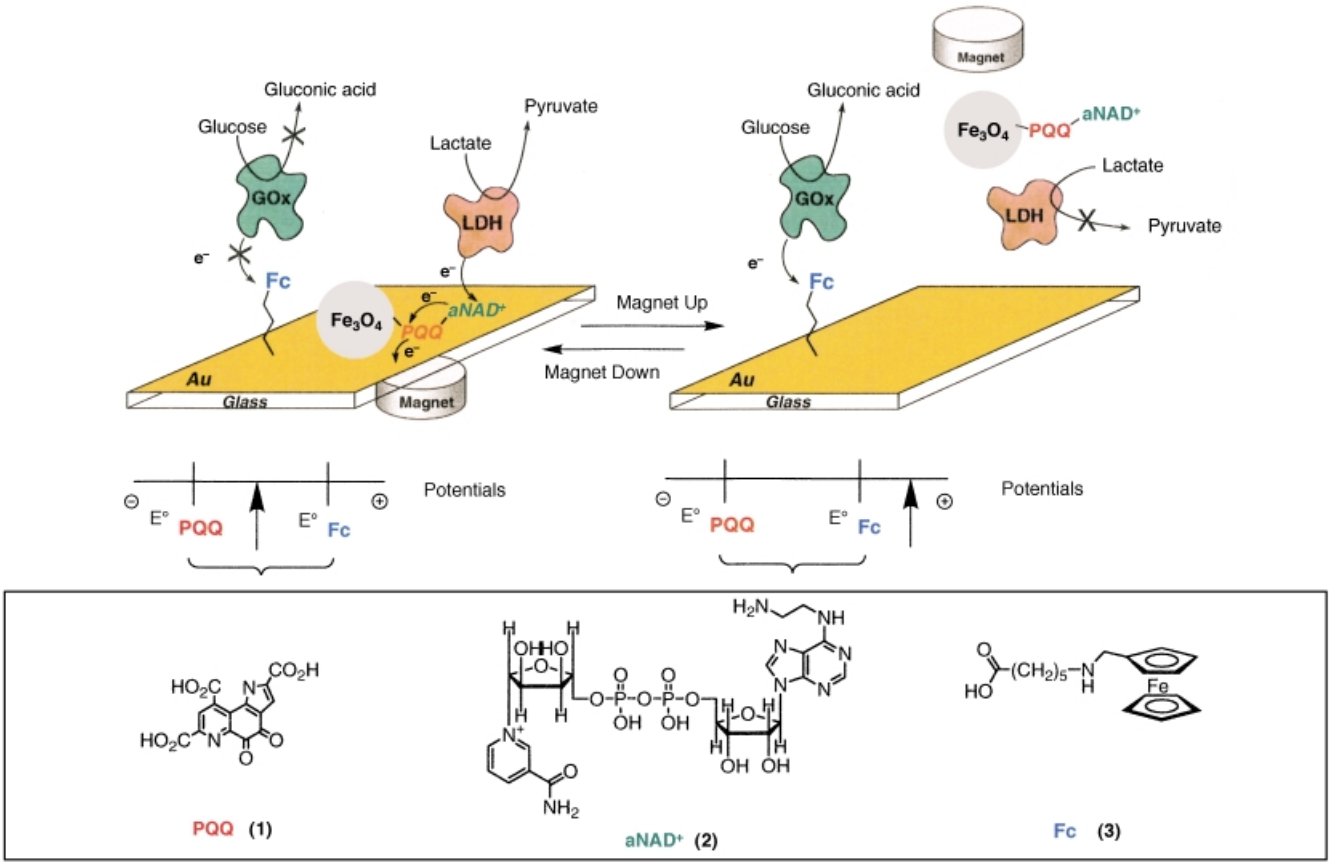

Figure 1. Magneto-switched dual biosensing of glucose and lactate.

  1433-7851/02/4108-1344 $ 20.00+.50/0 *Angew. Chem. Int. Ed.* **2002**, *41*, No. 8

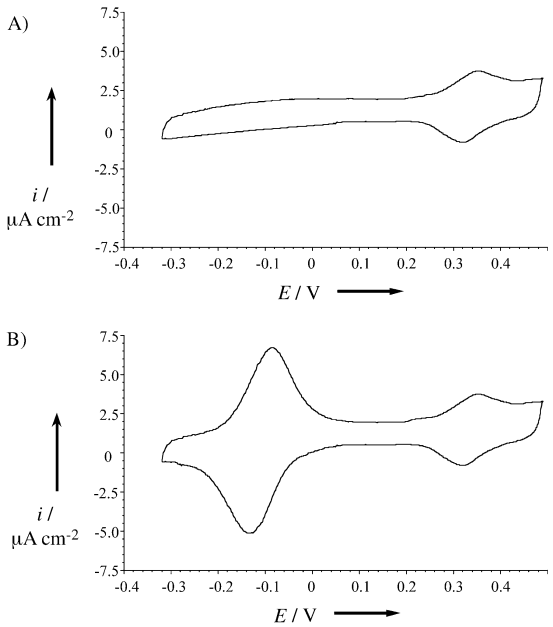

Figure 2. A) Cyclic voltammogram of the Fc-monolayer-functionalized Au electrode when the aNAD$^+$-PQQ-functionalized magnetic particles are retracted from the electrode by the external magnet. B) Cyclic voltammogram of the Fc-monolayer-functionalized Au electrode in the presence of the aNAD$^+$-PQQ-functionalized magnetic particles attracted to the electrode by the magnet. The data were recorded under argon in the presence of the magnetic particles, 20 mg, in 0.1 M Tris-HCl buffer, pH 7.0, $CaCl_2$, 10 mM. Potential scan rate, 100 mV s^{-1}.

process of the Fc monolayer is observed, $E^\circ = 0.32$ V. Switching the magnet to a position below the working electrode attracts the magnetic particles to the surface, which results in the generation of the characteristic redox wave of the PQQ units, $E^\circ = -0.13$ V (pH 7.0), and the redox wave of the Fc monolayer associated with the electrode ($E^\circ = 0.32$ V, Figure 2B). By cyclic positioning of the external magnet above the electrochemical cell or below the electrode, the functionalized magnetic particles may be reversibly drawn away from the electrode or attracted to the electrode, respectively. The electrical response of the system is then cycled between the single redox wave of the Fc-monolayer-functionalized electrode, and the two redox waves corresponding to the aNAD$^+$-PQQ-modified magnetic particles and the Fc-monolayer, respectively. The cyclic voltammograms shown in Figure 2 clearly reveal two potential regions where the detection of lactate or glucose may be exploited. When the magnet is positioned above the electrode and only the Fc units immobilized at the electrode are electrochemically active, the ferrocene-mediated biocatalytic oxidation of glucose can be achieved at potentials more positive than 0.32 V. When the magnet is positioned below the electrode and the aNAD$^+$-PQQ-functionalized magnetic particles are attracted to the electrode, the PQQ-mediated biocatalytic oxidation of lactate can be achieved in the potential region $-0.13\ \text{V} < E < 0.32$ V. It should be noted that when the magnet is positioned below the electrode the two bioelectrocatalytic processes, oxidation of glucose and of lactate, will occur at the potentials more positive than 0.32 V, where both electron mediators, PQQ and ferrocene, are oxidized.

Figure 3A, curve b, shows the cyclic voltammogram observed in the system consisting of the functionalized particles, GOx and LDH, and the two substrates, lactate and glucose, in the potential range $-0.1\ \text{V} - +0.6$ V, when the magnet is positioned above the cell. An electrocatalytic current corresponding to the Fc-mediated bioelectrocatalyzed oxidation of glucose by GOx is observed. Figure 3A, top (curve 1), shows the calibration curve corresponding to the amperometric responses of the system at variable concentrations of glucose. In this configuration the system is not sensitive to lactate in the entire concentration-range of glucose analysis, Figure 3A, top (curve 2). Figure 3B, curve b, shows the voltammetric

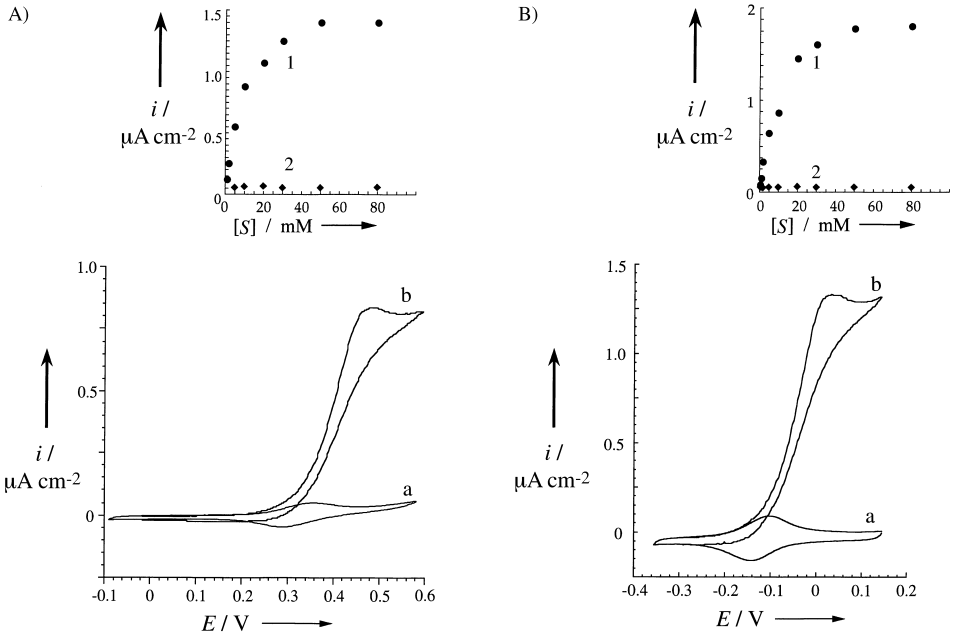

Figure 3. A) Bottom: cyclic voltammograms of the Fc-monolayer-functionalized Au electrode when the aNAD$^+$-PQQ-functionalized magnetic particles are retracted from the electrode by the external magnet: a) in the presence of GOx, 1 mg mL^{-1}, LDH, 2 mg mL^{-1}; b) in the presence of GOx, 1 mg mL^{-1}, LDH, 2 mg mL^{-1}, glucose, 50 mM, lactate, 20 mM. Top: Calibration plot of the amperometric response of the system with the magnet in the "up" position and the applied potential $E = 0.50$ V: 1) at different concentrations of glucose; 2) at different concentrations of lactate; B) bottom: cyclic voltammograms of the Fc-monolayer-functionalized Au electrode in the presence of the aNAD$^+$-PQQ-functionalized magnetic particles attracted to the electrode by the magnet: a) in the presence of GOx, 1 mg mL^{-1}, LDH, 2 mg mL^{-1}; b) in the presence of GOx, 1 mg mL^{-1}, LDH, 2 mg mL^{-1}, glucose, 50 mM, lactate, 20 mM. Top: Calibration plot of the amperometric responses of the system with the magnet in the "down" position and the applied potential $E = 0.05$ V: 1) at different concentrations of lactate; 2) at different concentrations of glucose. The data were recorded under argon in the presence of the magnetic particles (20 mg) in 0.1 M Tris-HCl buffer, pH 7.0, $CaCl_2$, 10 mM. Potential scan rate, 5 mV s^{-1}.

  1433-7851/02/4108-1345 $ 20.00+.50/0

response of the same system upon shifting the magnet to below the electrochemical cell and recording the electrochemical response of the system in the potential range -0.36 V– $+0.15$ V. An electrocatalytic anodic current is observed at the potentials where PQQ is oxidized. Lactate reduces the NAD^+-cofactor associated with the magnetic particles to NADH in the presence of LDH. The NADH is oxidized by PQQ which results in the formation of $PQQH_2$ and the regeneration of NAD^+. The magnetic particles attracted to the electrode by the external magnet actuate the electrochemical oxidation of $PQQH_2$, which results in the formation of the electrocatalytic anodic current. Figure 3B, top (curve 1), shows the calibration curve corresponding to the amperometric responses of the system at variable concentrations of lactate. It should be noted that in the applied potential-region the system is not sensitive to glucose in the entire concentration-range, Figure 3B, top (curve 2). The analysis of lactate can be switched on and off by shifting the magnet to positions below and above the working electrode. The selective analysis of each of the substrates by the two biocatalytic systems is impossible without the separation or blocking of one of the catalytic systems. Thus, the selective oxidation of lactate is accomplished by the application of a potential range (-0.13 V $< E <$ 0.32 V) that stimulates the bioelectrocatalyzed oxidation of lactate, but is not appropriate to activate the bioelectrocatalyzed oxidation of glucose. The selective oxidation of glucose ($E > 0.32$ V) is then achieved upon the magnetic-field-induced retraction of the $aNAD^+$-PQQ-functionalized magnetic particles from the electrode, a process that blocks the bioelectrocatalyzed oxidation of lactate. Thus, the selective analysis of each of the substrates is reversibly accomplished by limiting the potential to a range that is appropriate to activate only one bioelectrocatalytic system, and by the physical separation and blocking of one biocatalytic system using an external magnetic field.

In conclusion, we have demonstrated the use of a relay-modified-electrode, two enzymes, and functional magnetic particles as an integrated system for the programmed analysis of one of two substrates using an external magnet as an actuator.

Experimental Section

Magnetic particles (Fe_3O_4, saturation magnetization ca. 65 emu g^{-1}), approximately 1 μm average diameter, were prepared according to the published procedure[15] without including the surfactant into the reaction medium. The magnetic particles (500 mg) were silanized with [3-(2-aminoethyl)aminopropyl]trimethoxysilane, 5% (v/v), in dry toluene for 2 h under reflux.[7] Pyrroloquinoline quinone (PQQ; **1**, 1×10^{-3} M), was covalently linked to the amino-functionalized magnetic particles (200 mg) using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC; 1×10^{-2} M), as a coupling reagent in 0.1M HEPES-buffer, pH 7.2.[11a] Aminoethyl-functionalized NAD^+ ($aNAD^+$; **2**, 1×10^{-3} M) was covalently linked to the carboxylic groups of the PQQ bound to magnetic particles (200 mg) in the presence of EDC (1×10^{-2} M) in HEPES-buffer (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; 0.1M) pH 7.2.[13] The Au-electrode surface (ca. 2 cm^2 area) was modified with a monolayer of cystamine,[11a] and the ferrocene carboxylic acid derivative (Fc; **3**, 1×10^{-3} M) was covalently linked to the amino groups of the cystamine monolayer in the presence of EDC, 1×10^{-2} M, in HEPES-buffer (0.1M), pH 7.2.[16] The $aNAD^+$-PQQ-modified magnetic particles (20 mg) were introduced into an electrochemical cell that included the Fc-modified Au-working-electrode, glassy carbon counter electrode and saturated calomel reference electrode (SCE). The potentials in the paper are reported versus SCE. A 5 mm diameter NdFeB/Zn-coated magnet with the remanent magnetization of 10.8 kG was used to move the magnetic particles up and down. The electrochemical measurements were performed using an electrochemical analyzer (EG&G, model 6310) under argon at room temperature.

Received: August 30, 2001
Revised: January 24, 2002 [Z17824]

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