LETTER

Dual Magnetobiochemical Logic Control of Electrochemical Processes Based on Local Interfacial pH Changes

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ABSTRACT An electrode surface modified with a pH-sensitive polymeric brush was reversibly activated by local pH changes produced in situ by glucose oxidase associated with magnetic nanoparticles confined at the surface in the presence of an external magnet. The system mimics Boolean **AND** logic gate, with the magnetic and chemical input signals stimulating the electrochemical reactions at the switchable interface. Biomedical applications of the "smart" interface controlled by enzymatic reactions through local pH changes are anticipated in various implantable biomedical devices.

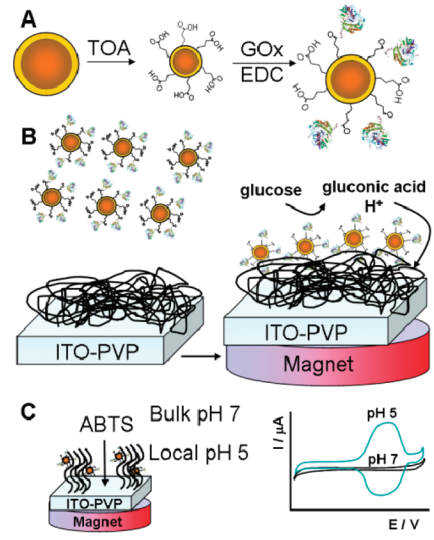
KEYWORDS: switchable interface • local pH change • enzyme • modified electrode • magnetic nanoparticles • logic gate

Functional interfaces with switchable and tunable properties (1) have been developed as components of various bio/nanocomposite electronic systems including biosensors (2), fuel cells (3, 4), information processing units (5), etc. Optical, magnetic, electrical, and chemical signals were applied to trigger ON−OFF switching of the electrochemical interfaces (6). Unconventional chemical computing was accomplished using various logic systems composed of chemical (7−9) and biomolecular components (10).

Recently pioneered enzyme logic gates (11, 12) were used to switch ON−OFF an electrode activity by logically processed biochemical signals (13). Biologically inspired scaling-up complexity of the chemical signal-processing systems resulted in the development of sophisticated logic networks controlling an electrode activity (14). Application of chemical information-processing systems for the control of electrochemical/electronic devices requires signal-responsive materials for mediating and amplification of biochemically generated stimuli output signals (15). Recently developed electrochemical systems controlled by enzyme logic networks were based on pH changes generated in a bulk solution by biochemical reactions, resulting in restructuring of polymer layers on electrodes and producing ON−OFF states at the modified interfaces (13, 14). The present paper addresses for the first time application of local interfacial pH changes generated by a bionanocomposite system to trigger switching processes at an electrode surface.

Au shell/$CoFe_2O_4$ core nanoparticles (NPs; 18 ± 3 nm diameter, 70 emu g^{-1} specific magnetization) (16) were functionalized with glucose oxidase (GOx, from *Aspergillus niger*, type X-S, E.C.1.1.3.4) and used for magnetoassisted translocation of the enzyme. The Au shell was functionalized with α-lipoic acid (TOA), and the carboxylic groups of the self-assembled monolayer were used for carbodiimide coupling of the enzyme (ca. 4 GOx per NP) (Scheme 1A) (details are given in the Supporting Information). An indium−tin oxide (ITO) electrode (geometrical area, 0.4 cm^2; surface roughness factor, ca. 1.6 ± 0.1; surface resistivity, 20 ± 5 Ω/sq) was modified with a poly(4-vinylpyridine) (P4VP; MW 160 kDa) polymer brush (ca. 0.075 chains per nm^2) (details

Scheme 1. (A) Functionalization of Au Shell/$CoFe_2O_4$ Magnetic-Core NPs with GOx, (B) Magnetoassisted Concentration of the GOx-NPs on the Electrode Surface Modified with the P4VP Brush To Perform Glucose Oxidation at the Interface, and (C) Opening of the P4VP Brush for the Electrochemical Reaction at Acidic pH Generated at the Interface upon Biocatalytic Reaction



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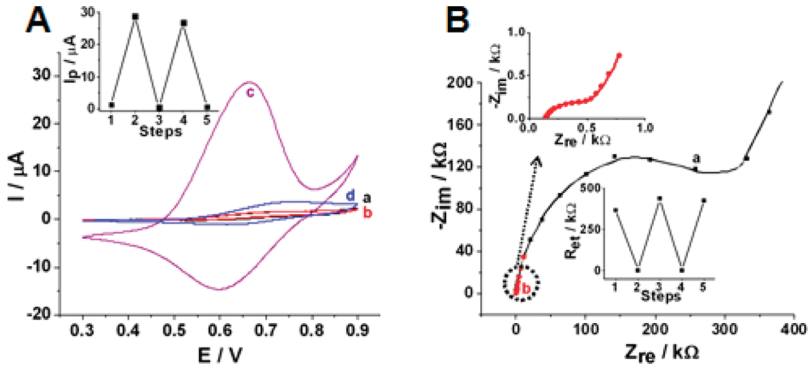

FIGURE 1. (A) Cyclic voltammograms obtained on the P4VP-modified electrode: (a) GOx-NPs in the solution in the absence of glucose; (b) GOx-NPs confined at the electrode in the absence of glucose; (c) GOx-NPs confined at the electrode in the presence of glucose; (d) GOx-NPs redispersed in the solution in the presence of glucose. Inset: reversible ON−OFF electrode switching by adding and removing glucose while the GOx-NPs are confined at the electrode surface. (B) Impedance spectra (Nyquist plots) obtained on the P4 VP-modified electrode with the GOx-NPs confined at the electrode: (a) in the absence of glucose; (b) in the presence of glucose (also shown at a smaller scale). Inset: reversible switching of R_{et} by adding and removing glucose. Solution: 0.1 mM ABTS in 0.1 M Na_2SO_4, pH 7; GOx-NPs, 0.3 mg mL^{-1}; glucose addition, 10 mM; cyclic voltammograms, 100 mV s^{-1}; impedance bias potential, 0.62 V.

are given in the Supporting Information) (17). The P4VP-modified electrode reveals pH-dependent electrochemical activity due to reversible restructuring of the polymer thin film between a swollen hydrophilic state with the protonated polymer chains (pH < 4.5) and a shrunken hydrophobic state with the neutral polymer (pH > 6.5) (17). The swollen polymer brush is permeable for 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; 0.1 mM), used as a soluble redox probe, while the shrunken state of the polymer completely insulates the electrode surface, thus fully inhibiting the redox process. The pH changes resulting in the switching of the interfacial properties between the OFF and ON states were generated in situ upon oxidation of glucose biocatalyzed by GOx that resulted in the formation of gluconic acid.

In order to generate the pH changes in close vicinity to the electrode surface, GOx was transported to the interface with the magnetic NPs, 0.3 mg mL^{-1} (Scheme 1B). The GOx-NPs were reversibly attracted to the electrode surface and removed from it upon application of an external magnetic field (NdFeB magnet). The magnetic field measured in the center of the electrode was 2.5 T, resulting in a collection of ca. 90% of the GOx-NPs at the electrode surface (based on the optical measurements in the solution). In both positions of the GOx-NPs (localized at the electrode surface and dispersed in the bulk solution), we added glucose (10 mM) to the solution to activate the biocatalytic process. The experiment was always started at pH 7 (0.1 M Na_2SO_4) when the modified electrode is in the OFF state, and the changes at the electrode surface were monitored by cyclic voltammetry and Faradaic impedance spectroscopy (Figure 1). When the GOx-NPs were dispersed in the bulk solution (regardless of the presence or absence of glucose), the electrode demonstrated the OFF state characterized by small currents in the cyclic voltammogram (Figure 1A, curve a). It should be noted that in the time scale of the experiment (210 min) no significant pH changes in the solution were observed (ΔpH < 0.7) when glucose was added to the bulk dispersion of the GOx-NPs. The magnetoassisted collection of the GOx-NPs at the electrode surface did not result in any changes in the cyclic voltammogram if glucose was not added to the solution (Figures 1, curve b). A large electron-transfer resistance, R_{et} (ca. 350 kΩ), derived from the impedance spectrum (Figure 1B, curve a) was also observed in this case. The addition of glucose to the solution when the GOx-NPs were concentrated on the electrode surface resulted in the opening the electrode surface, and a reversible cyclic voltammogram was observed for the ABTS redox probe after 210 min of biocatalytic reaction (Figure 1A, curve c). Simultaneously, R_{et} was decreased to a much smaller value of ca. 700 Ω (Figure 1B, curve b; shown also in a small scale), reflecting the ON state of the electrode. The pH value of the bulk solution was almost unaffected by the biocatalytic reaction proceeding at the electrode surface. Removing the glucose solution from the cell and substituting it with a background solution without glucose resulted in the return of the electrode to the OFF state with a low current in the cyclic voltammogram and a high R_{et} in the impedance spectrum. Cyclic addition−removal of glucose to and from the solution resulted in the reversible switching ON−OFF of the electrode activity followed by cyclic voltammetry and impedance spectroscopy (Figure 1, insets). Finally, the GOx-NPs were removed from the electrode surface by the magnet and redispersed in the bulk solution. This resulted in the OFF state of the electrode (Figure 1A, curve d). After that, the addition of glucose to the bulk suspension of the GOx-NPs did not result in electrode activation. Thus, the electrode was activated only if the GOx-NPs were concentrated on the surface with the help of an external magnet and glucose was added to the solution (Scheme 1C). The input signals included application of the external magnetic field and the addition of glucose to perform an **AND** logic operation at the electrode surface, resulting in electrode activation. Minor variations of the current and interfacial resistance values in the OFF state of the electrode originated from capacitance changes (upon attraction/retraction of the magnetic nanoparticles) and slight changes of the pH value in the unbuffered solution.

In order to prove that activation of the electrode originates from local pH changes produced by biocatalytic reac-

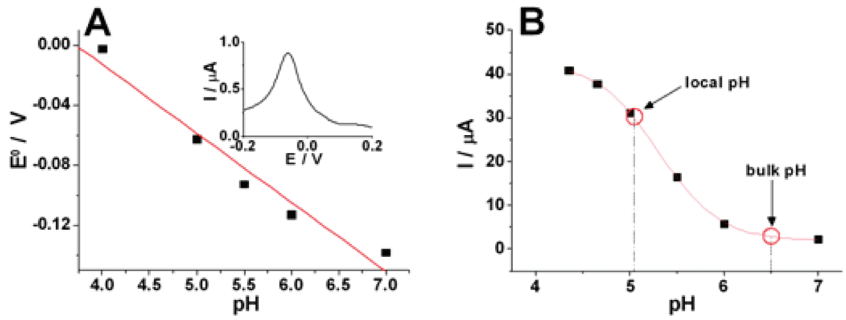

FIGURE 2. (A) pH dependence of the thionin E^0 potential measured at a bare ITO electrode. Inset: Differential pulse voltammogram obtained for thionin (0.1 mM) at the P4VP electrode with the confined GOx-NPs in the presence of glucose (10 mM). (B) Titration curve for the P4VP electrode measured by cyclic voltammetry in the presence of ABTS (0.1 mM). Highlighted points show the bulk and local pH values generated in the presence of the surface-confined GOx-NPs and glucose (10 mM).

tion, we applied thionin (0.1 mM) as a redox probe with a pH-dependent redox potential (Figure 2A). The redox potential of thionin measured at the ON state of the electrode produced by the GOx-NPs in the presence of glucose was −0.06 V (vs Ag|AgCl|KCl 3 M) (Figure 2A, inset), which corresponds to that of the local interfacial pH 5.05. It should be noted that during this experiment the bulk pH value was ca. 6.5. We also measured the ABTS potential peak current by cyclic voltammetry in a 100 mM phosphate buffer titrated to different pH values (Figure 2B). The obtained dependence of the peak current versus pH reflects the transition of the electrode surface from the OFF state (pH > 6) to the ON state (pH < 4.5). Placing the local interfacial pH value derived from the redox potential of thionin on the titration curve of the electrode, we can conclude that the biocatalytic process induced by the GOx-NPs at the interface results in the opening of the electrode by ca. 70%. At the same time, the bulk pH value corresponds to the fully closed electrode surface. Similar results were obtained when a weak buffer (HEPES, 8 mM, pH 7.4) mimicking the buffer properties of blood was used as the background solution. It should be noted that additions of H_2O_2 (another product of the biocatalytic reaction) to the solution at pH 6.5 in a control experiment did not result in the pH changes and activation of the electrode interface.

The present results demonstrate the possibility of controlling the activity of switchable surfaces by producing local pH changes upon biocatalytic reactions running at the interfaces. The external signals activating the interfaces might be chemical (e.g., glucose) or physical (magnetic field) or both of them together (**AND** logic gate). The complexity of the biocatalytic system processing the biochemical signals can be scaled up to operate with many different signals processed by various logic operations performed by an enzyme logic network associated with the switchable interface (14). We anticipate that this approach will find numerous applications in future "smart" implantable biomedical devices responding to the current physiological needs of a patient. The local pH changes produced by biochemical reactions will control the operation of implanted biomedical systems without the need for bulk changes in the physiological environment.

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Supporting Information Available: Preparation and characterization of the GOx-NPs and P4VP-electrode. This material is available free of charge via the Internet at http://pubs.acs.org.

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