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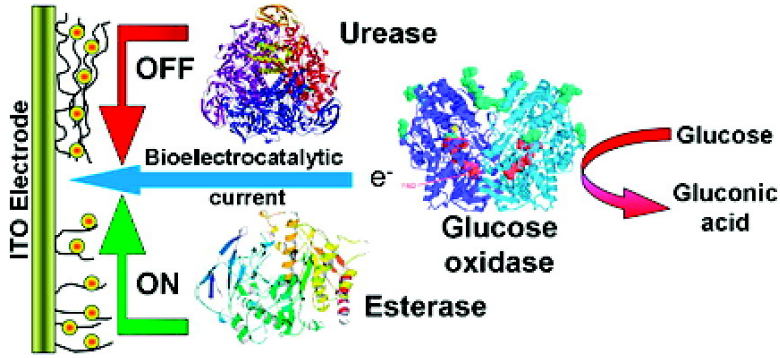

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Biochemically Controlled Bioelectrocatalytic Interface

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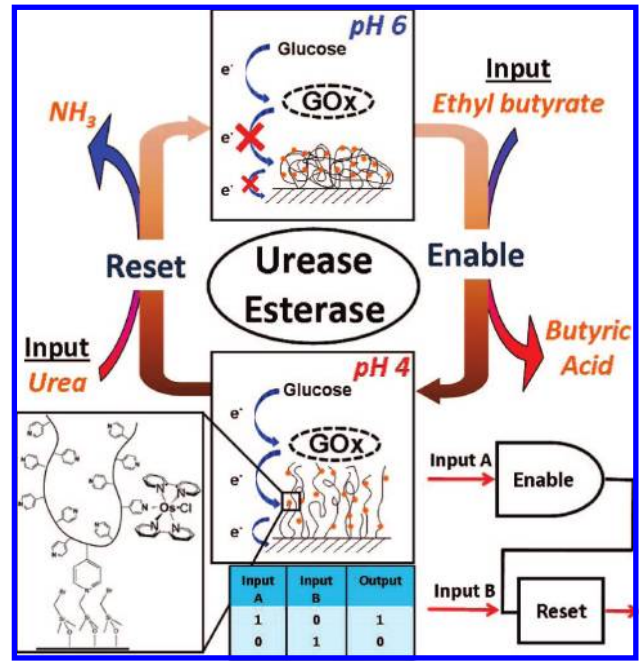
Natural biochemical processes are controlled and regulated by different mechanisms at various levels of complexity involving numerous biochemical and physiological reactions.[1] Artificial biocatalytic and particularly bioelectrocatalytic processes used in various bioelectronic devices, such as biosensors, biofuel cells, and bioactuators, are suffering from the lack of the biochemical control and regulation. Recently developed switchable and tunable bioelectrocatalytic systems are controlled by various physical input signals: electrical,[2] magnetic,[3] light,[4] etc. allowing their applications in novel biosensors[5] and biofuel cells[6] with an adjustable performance. However, this approach does not provide biochemical/physiological control over their operation. There is an obvious need of another kind of their functional regulation based on an interface between bioelectronic systems and their biochemical environment. The present paper addresses the challenging aim of biochemical control over bioelectronic systems by integrating a bioelectrocatalytic system with a stimuli-responsive material and enzyme-catalyzed stimuli-generating reactions.

Numerous stimuli-responsive materials sensitive to a big variety of external physical/chemical signals have been developed for various applications, particularly when they are assembled at functional interfaces.[7] An important part of them is based on pH-controlled polyelectrolytes, which are switchable between charged swollen and uncharged shrunken states upon protonation/deprotonation processes controlled by a solution pH value.[8] Recently electrochemical gates based on nanostructured polymer brushes[9] or membranes,[10] made of pH-switchable polyelectrolytes, were developed to control electrode interfacial properties by external pH changes. Incorporation of redox species into pH-sensitive polymer brushes resulted in a pH-switchable electrode capable of pH-controlled bioelectrocatalytic reactions.[11] This system was one step away from bioelectrocatalytic systems controlled by external biochemical processes. The last step to assemble a bioelectrocatalytic system controlled by a set of biochemical reactions is described below.

The modified electrode was prepared by grafting a poly(4-vinyl pyridine) (P4VP) brush functionalized with $Os(dmo\text{-}bpy)_2^{2+}$ (dmo-bpy = 4,4′-dimethoxy-2,2′-bipyridine) redox groups on an ITO electrode.[11] A low surface loading of the polymer, ca. 0.075 chain·nm^{-2}, allowed quasi-diffusional motion of the polymer chains providing direct electrical contacting between the redox groups associated with the polymer (ca. 4 Os complexes per one P4VP chain) and the electrode support, thus resulting in the reversible electrochemical process, $E° = 0.28$ V (vs Ag|AgCl|KCl 3 M, at pH 4.0). The electron exchange between the polymer-bound Os complex and the electrode support was allowed only when the polymer was in a swollen state due to the protonation of the pyridine groups at pH < 4.5. When the polymer was shrunken at its neutral state at pH > 5, the polymer chain motion was "frozen" and the electrochemical process was inhibited. The primary redox process of the surface-confined Os complex was coupled with the glucose oxidation biocatalyzed by soluble glucose oxidase (GOx) and mediated by the redox polymer. The system has demonstrated the pH-controlled switchable bioelectrocatalysis features being bioelectrocatalytically active at pH < 4.5 and mute at pH > 5.[11]

Scheme 1. Switchable Bioelectrocatalytic Oxidation of Glucose Controlled by External Enzymatic Reactions



We applied enzyme-catalyzed reactions to change the pH value *in situ*, thus resulting in the activation and inhibition of the pH-controlled bioelectrocatalytic oxidation of glucose, Scheme 1. The biochemically controlled bioelectrocatalytic system was composed of the Os-modified electrode, glucose (80 mM), and three soluble enzymes: glucose oxidase (GOx, *Aspergillus niger*, type X-S, E.C. 1.1.3.4, 150 units·mL^{-1}), esterase (Est, from *porcine liver*, E.C. 3.1.1.1, 5 units·mL^{-1}), and urease (Ur, from *jack beans*, E.C. 3.5.1.5, 5 units·mL^{-1}) in an aqueous solution including 0.01 M sodium sulfate. Oxygen was removed from the solution by Ar bubbling to prevent the natural electron pathway resulting in the formation of H_2O_2. The experiments started under the mute state of the bioelectrocatalytic system, pH ca. 6.5, when the polymer-modified electrode is redox inactive and not able to mediate the electron transport from GOx to the conductive support, even with a 0.6 V potential applied constantly on the modified electrode. A cyclic voltammogram recorded at this state of the system (Figure 1, curve a) does not show any Faradaic current, and an impedance spectrum (Figure 2, curve a) demonstrates a high electron transfer resistance (ca. 2.4 MΩ).

To activate the redox process and enable the bioelectrocatalytic oxidation of glucose, ethyl butyrate, 10 mM, was added to the

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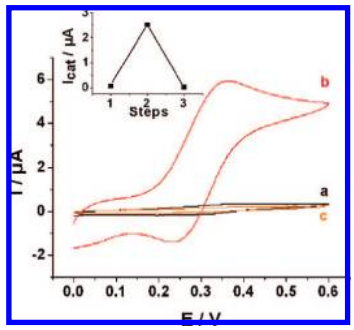

Figure 1. Cyclic voltammograms obtained for the switchable bioelectrocatalytic glucose oxidation when the system is (a) in the initial OFF state, pH ca. 6.5; (b) enabled by the ethyl butyrate input signal, pH ca. 3.8; and (c) inhibited by the urea reset signal, pH ca. 7.5. Scan rate, 10 mV s^{-1}. Inset: Switchable bioelectrocatalytic current: (step 1) initial OFF state, (step 2) enabled ON state, (step 3) reset to the OFF state.

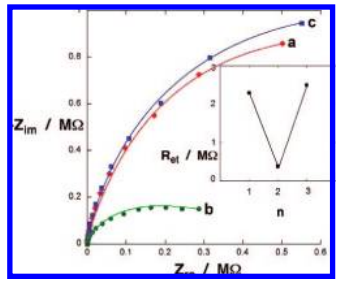

Figure 2. Impedance spectra obtained for the switchable bioelectrocatalytic glucose oxidation when the system is (a) in the initial OFF state, pH ca. 6.5; (b) enabled by the ethyl butyrate input signal, pH ca. 3.8; (c) inhibited by the urea reset signal, pH ca. 7.5. Inset: Switchable electron transfer resistance: (step 1) initial OFF state, (step 2) enabled ON state, (step 3) reset to the OFF state.

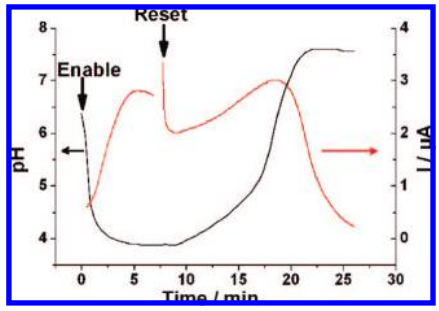

Figure 3. Time-dependent pH value and electrocatalytic current (at 0.6 V) measured *in situ* upon the Enable and Reset signals applied to the bioelectrocatalytic system.

solution, Scheme 1. This resulted in the production of butyric acid biocatalyzed by Est, thus resulting in the lowering of the solution pH value and activation of the modified electrode, therefore enabling the biocatalytic oxidation of glucose. The pH value of the solution and the biocatalytic anodic current corresponding to the glucose oxidation were followed during the process (Figure 3). When the solution pH reached the minimum value of ca. 3.8 and the maximum anodic current was observed, we recorded a cyclic voltammogram (Figure 1, curve b) demonstrating the bioelectrocatalytic wave, which corresponded to the oxidation of glucose biocatalyzed by GOx and mediated by the modified electrode being in the active state. The impedance spectrum recorded at this state demonstrated a low electron transfer resistance, 0.26 MΩ, correlating with the enabled bioelectrocatalytic process (Figure 2, curve b). Then, urea, 2 mM, was added to the solution to reset the initial pH in the system and to switch off the bioelectrocatalytic process, Scheme 1. This resulted in the formation of ammonia biocatalyzed by urease, thus increasing the pH value of the solution and inhibiting the bioelectrocatalytic process (Figure 3). The cyclic voltammogram and impedance spectrum recorded after the pH was reset have demonstrated the mute state of the bioelectrocatalytic system (Figures 1 and 2, curve c). The reversible transformations exemplified in Figures 1 and 2, insets, can be repeated several times without substantial degradation of the system. Considering additions of ethyl butyrate and urea as input signals A and B, we could present a simple equivalent electronic scheme and a truth table corresponding to the electronic description of the switchable bioelectrocatalytic process.

The present simple system aims to demonstrate the concept of the biochemical control over bioelectronic systems. It should be noted that recently designed enzyme-based logic gates[12] could be assembled into multicomponent assemblies[13] similarly to biochemical pathways allowing biochemical processing of complex information. Integration of the biocomputing enzyme-based systems with bioelectronic devices based on signal-responsive materials would allow biochemical (ultimately physiological) control over the bioelectronic systems. "Smart" biochemically controlled bioelectronic devices for biosensor, biofuel cell, and bioactiating applications are envisaged based on the described approach. To reach this exciting aim, further work on the optimization of the enzyme-logic systems is needed.[14]

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Supporting Information Available: The experimental details on the bioelectrocatalytic system are given. This material is available free of charge via the Internet at http://pubs.acs.org.

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