J|A|C|S COMMUNICATIONS

Published on Web 07/31/2009

Biofuel Cell Logically Controlled by Antigen−Antibody Recognition: Towards Immune-Regulated Bioelectronic Devices

Tsz Kin Tam, Guinevere Strack, Marcos Pita, and Evgeny Katz*

Department of Chemistry and Biomolecular Science, and NanoBio Laboratory (NABLAB), Clarkson University, Potsdam, New York 13699-5810

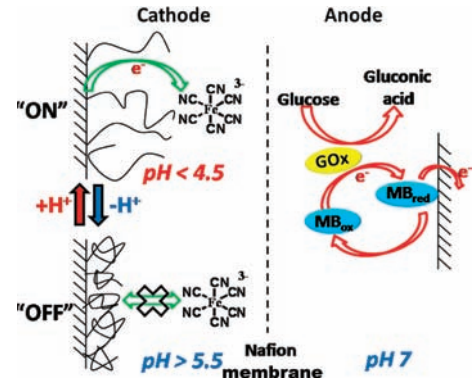
Received June 13, 2009; E-mail: ekatz@clarkson.edu

Microbial[1] and enzyme-based[2] biofuel cells recently attracted attention for being considered as potential sources of sustainable energy. Such systems might be miniaturized and applied as implantable energy sources for biomedical applications.[3] In addition to major research activity aiming at increasing the energy efficiency and lifetime of biofuel cells,[4] some interesting results were recently reported for biofuel cells with controlled power release.[5] The biofuel cells with switchable/tunable activity were developed using switchable biocatalytic electrodes controlled by an external electrical[6] or magnetic[7] signal. Recently pioneered enzyme logic gates[8] processing biochemical signals according to built-in Boolean logic resulted in the development of biocatalytic electrodes controlled by logic gates[9] or their networks.[10] Experimental and theoretical work on the networking of enzyme logic gates promises to scale up the complexity of biocomputing systems based on enzyme catalyzed reactions with a final aim of constructing artificial systems comparable with natural biochemical pathways.[11] However, even currently available systems of relatively low complexity are considered as promising multisignal-controlled biosensors[12] or actuators[13] for biomedical applications. This approach applied to biofuel cells allowed for energy release controlled by logically processed biochemical signals.[14]

Enzyme-based logic gates[8] represent one of the options in the general area of biocomputing.[15] Application of DNA[16] or eventually whole biological cells[17] for processing biochemical information resulted in the broadening of the biocomputing concept. However, to the best of our knowledge, immune reactions were not applied yet for the biochemical information processing. Their application might significantly increase the versatility of the biocomputing systems resulting in very specific responses to a large variety of biochemical signals. Coupling switchable biofuel cells with immune-controlled logic systems would enhance the adaptability of future implantable bioelectronic devices to physiological conditions. The present paper reports on the first example of an immune-controlled biofuel cell processing signals of antibodies according to the built-in Boolean **NOR** logic gate.

A simple model biofuel cell was composed of two indium tin oxide electrodes (ITO coated glass, 1.2 cm^2 geometrical area, 20 $\pm$ 5 Ω/sq surface resistivity, Aldrich). The cathode was modified with a pH-switchable poly(4-vinyl pyridine) (P4VP) polymer brush[18] (see details in the Supporting Information) operating in the presence of 10 mM $K_3[Fe(CN)_6]$ used as a model oxidizer in a nonbuffered solution of 100 mM Na_2SO_4. The anode was an unmodified ITO electrode operating in the presence of soluble glucose oxidase (GOx, type X-S from *Aspergillus niger* (E.C. 1.1.3.4); 250 units mL^{-1}) which oxidized glucose fuel, 100 mM, with the help of a diffusional redox mediator methylene blue, 0.1 mM in 100 mM phosphate buffer, pH 7.0, under Ar. The electrodes were separated with a Nafion membrane (0.09 mm thick, Alfa Aesar). The oversimplified design of the biofuel cell, Scheme 1, was specially selected to demonstrate clearly the power control by the immune-based logic gate without any complications from secondary effects related to the bioelectrocatalytic reactions.

Scheme 1. pH-Switchable Biofuel Cell for Controlling the Power Release by Immune Signals

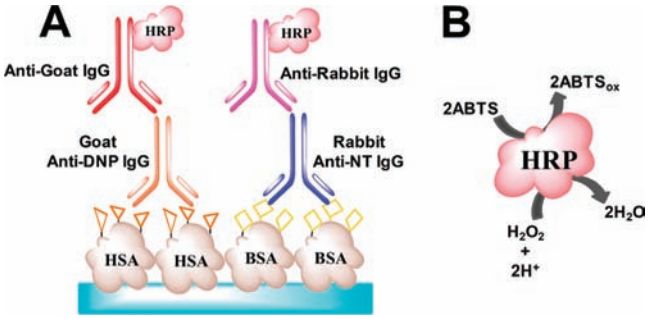


The immune-system mimicking Boolean **NOR** logic operation was composed of a polystyrene support modified with a mixture of 2,4-dinitrophenyl (DNP) and 3-nitro-L-tyrosine (NT) antigens coupled with human serum albumin (HSA) and bovine serum albumin (BSA), respectively, Scheme 2A (see details in the Supporting Information). Primary antibodies, anti-DNP (antidinitrophenyl IgG polyclonal from goat, Oxford Biomedical) and anti-NT (antinitrotyrosine IgG from rabbit, Sigma-Aldrich), were applied as input signals (**A** and **B** signals, respectively) to the antigen-functionalized surface. The absence of the antibodies was considered as the logic input **0**, while their optimized concentrations at 8 $\mu g \cdot mL^{-1}$ for anti-DNP and 0.2 $\mu g \cdot mL^{-1}$ for anti-NT were defined as logic input **1**. The immune-input signal concentrations for the logic **1** value were optimized to produce similar effects on the biofuel cell. The input signals were applied in all four combinations (**0**,**0**; **0**,**1**; **1**,**0**; and **1**,**1**, where the first notation corresponds to signal **A** and the second to signal **B**) and reacted with the modified surface. After reacting the antigen-functionalized surfaces with the antibody signals, the surfaces were treated with a mixture of secondary antibodies, antigoat-IgG-HRP and antirabbit-IgG-HRP (mouse origin IgG against goat immunoglobulin and mouse origin IgG against rabbit IgG, both labeled with HRP, from Jackson Immuno, 0.05 $mg \cdot mL^{-1}$ each antibody), to attach the biocatalytic HRP tags to the immune complexes generated on the surfaces. The modified interface was characterized by AFM, and sequential assembling of the layered immune system was confirmed for **0**,**1**; **1**,**0**; and **1**,**1** combinations of the antibodies input signals, while the molecular density in the assembly was almost double in the case of **1**,**1** signals compared with **0**,**1** and **1**,**0** signal combinations. Then the functionalized surfaces were treated with 0.5 mM 2,2′-azino-bis(3-

10.1021/ja9048459 CCC: $40.75 © 2009 American Chemical Society

ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 0.5 mM H_2O_2 producing different results depending on the applied combination of the primary antibody-input signals, Scheme 2B.

Scheme 2[a]



[a] (A) The immune system producing *in situ* pH changes to control the power production by the switchable biofuel cell. (B) The biocatalytic reaction resulting in the pH changes.

The biocatalytic HRP tag bound to the secondary antibodies appeared on the functionalized surface only if at least one of the primary antibodies was bound there. Thus, the surface stayed biocatalytically inactive (without surface confined HRP) upon application of **0**,**0** input signals of the primary antibodies, yielding no ABTS oxidation and no pH changes in the solution. When the input signal combinations **0**,**1**; **1**,**0**; and **1**,**1** were applied, the secondary antibodies and the associated HRP tag were bound through one or both primary antibodies on the surface, Scheme 2A. This resulted in the biocatalytic oxidation of ABTS in the solution. The biocatalytic process was also accompanied by the respective increase of pH value starting from the initial value of ca. 4.5 and reaching pH ca. 5.8 in 180 min (see details in the Supporting Information). The biocatalytic reaction was performed *in situ* in the cathodic compartment of the biofuel cell affecting the electrochemical process at the pH-switchable P4VP-modified electrode. This electrode was shown to be reversibly switched between ON and OFF states due to the swelling and shrinking of the polymer brush at pH < 4.5 and pH > 5.5, respectively.[10,18] The experiments with the biofuel cell were always started at pH ca. 4.5 in the cathodic compartment when the P4VP-electrode was in the ON state and the biofuel cell produced a high power output. Application of **0**,**0** input signals did not change the power output of the cell, Figure 1A,B, curves a. When **0**,**1**; **1**,**0**; and **1**,**1** immune-input signals were applied, the pH was increased up to 5.8, thus resulting in the OFF state of the P4VP-modified electrode, also switching the biofuel cell OFF, Figure 1A,B, curves b. The biofuel cell operation controlled by the immune signals resembles the **NOR** logic function, Figure 1B, inset. It should be noted that the OFF state of the cell revealed some residual activity because the pH change generated *in situ* was not enough for the full transition of the P4VP-electrode to the OFF state; however the biofuel cell activity decreased substantially. If the biofuel cell was switched OFF upon appropriate combinations of the immune-input signals, the cell activity was then restored by the **Reset** function. To activate the biofuel cell GOx, 14.3 units mL^{-1}, and glucose, 10 mM were added to the cathodic compartment, resulting in the pH decrease to ca. 4.2 in 10 min, thus reactivating the biofuel cell, Figure 1B, inset.

The studied biofuel cell demonstrated for the first time the possibility to control the power release by immune signals processed according to Boolean logic operations. This opens opportunities for future implantable bioelectronic devices logically controlled by

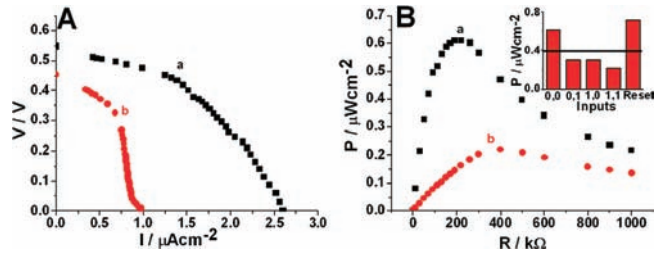


Figure 1. (A) Polarization curves of the biofuel cell in the ON (a) and OFF (b) states. (B) Power release on different load resistances in the ON (a) and OFF (b) states. Inset: Maximum power produced by the cell upon application of different combinations of the immune-input signals.

immune signals. Our preliminary results reported elsewhere[19] demonstrated switchable electrochemical reactions controlled by local interfacial pH changes rather than by bulk pH change. Implementation of this approach will result in the biofuel cells integrated with the immune-logic systems operating directly on the bioelectrocatalytic interfaces. Further scaling up of the complexity of biocomputing system controlling biofuel cell activity will be achieved by networking immune and enzyme-based logic gates responding to a large variety of biochemical signals.

Acknowledgment. NSF Grants DMR-0706209, CCF-0 726698 and SRC Award 2008-RJ-1839G are gratefully acknowledged. T.K.T. and G.S. acknowledge Wallace H. Coulter scholarships from Clarkson University.

Supporting Information Available: Preparation and characterization of the immobilized immune systems, pH changes produced by them, and synthesis of the P4VP-modified electrode. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Lu, N.; Zhou, S. G.; Ni, J. R. *Prog. Chem.* **2008**, *20*, 1233–1240. (b) Du, Z. W.; Li, H. R.; Gu, T. Y. *Biotechnol. Adv.* **2007**, *25*, 464–482. (c) Yi, G.; Xin, Z. *Prog. Chem.* **2007**, *19*, 74–79.
(2) (a) Cracknell, J. A.; Vincent, K. A.; Armstrong, F. A. *Chem. Rev.* **2008**, *108*, 2439–2461. (b) Minteer, S. D.; Liaw, B. Y.; Cooney, M. J. *Curr. Opin. Biotechnol.* **2007**, *18*, 228–234.
(3) (a) Barton, S. C.; Gallaway, J.; Atanassov, P. *Chem. Rev.* **2004**, *104*, 4867–4886. (b) Heller, A. *Anal. Bioanal. Chem.* **2006**, *385*, 469–473.
(4) Moehlenbrock, M. J.; Minteer, S. D. *Chem. Soc. Rev.* **2008**, *37*, 1188–1196.
(5) Katz, E. *Electroanalysis* **2010**, special issue on Biofuel Cells, in press.
(6) Katz, E.; Willner, I. *J. Am. Chem. Soc.* **2003**, *125*, 6803–6813.
(7) Wang, J.; Musameh, M.; Laocharoensuk, R.; Gonzalez-Garcia, O.; Oni, J.; Gervasio, D. *Electrochem. Commun.* **2006**, *8*, 1106–1110.
(8) (a) Strack, G.; Pita, M.; Ornatska, M.; Katz, E. *ChemBioChem* **2008**, *9*, 1260–1266. (b) Baron, R.; Lioubashevski, O.; Katz, E.; Niazov, T.; Willner, I. *J. Phys. Chem. A* **2006**, *110*, 8548–8553.
(9) Zhou, J.; Tam, T. K.; Pita, M.; Ornatska, M.; Minko, S.; Katz, E. *ACS Appl. Mater. Interfaces* **2009**, *1*, 144–149.
(10) Privman, M.; Tam, T. K.; Pita, M.; Katz, E. *J. Am. Chem. Soc.* **2009**, *131*, 1314–1321.
(11) (a) Privman, V.; Arugula, M. A.; Halámek, J.; Pita, M.; Katz, E. *J. Phys. Chem. B* **2009**, *113*, 5301–5310. (b) Privman, V.; Strack, G.; Solenov, D.; Pita, M.; Katz, E. *J. Phys. Chem. B* **2008**, *112*, 11777–11784.
(12) Manesh, K. M.; Halámek, J.; Pita, M.; Zhou, J.; Tam, T. K.; Santhosh, P.; Chuang, M.-C.; Windmiller, J. R.; Abidin, D.; Katz, E.; Wang, J. *Biosens. Bioelectron.* **2009**, *24*, 3569–3574.
(13) Tokarev, I.; Gopishetty, V.; Zhou, J.; Pita, M.; Motornov, M.; Katz, E.; Minko, S. *ACS Appl. Mater. Interfaces* **2009**, *1*, 532–536.
(14) Amir, L.; Tam, T. K.; Pita, M.; Meijler, M. M.; Alfonta, L.; Katz, E. *J. Am. Chem. Soc.* **2009**, *131*, 826–832.
(15) Shao, X. G.; Jiang, H. Y.; Cai, W. S. *Prog. Chem.* **2002**, *14*, 37–46.
(16) Ezziane, Z. *Nanotechnology* **2006**, *17*, R27–R39.
(17) Simpson, M. L.; Sayler, G. S.; Fleming, J. T.; Applegate, B. *Trends Biotechnol.* **2001**, *19*, 317–323.
(18) Tam, T. K.; Ornatska, M.; Pita, M.; Minko, S.; Katz, E. *J. Phys. Chem. C* **2008**, *112*, 8438–8445.
(19) Pita, M.; Tam, T. K.; Minko, S.; Katz, E. *ACS Appl. Mater. Interfaces* **2009**, *1*, 1166–1168.

JA9048459