

Harnessing self-organizing bacterial networks for microbial fuel cells

Ryuhei Nakamura and Kazuhito Hashimoto

Metal-reducing bacteria can construct electrically conductive networks using semiconductive nanominerals.

Present concerns about the depletion of natural resources have encouraged studies to exploit microbial ingenuity to harness renewable sources of energy.¹ Microbes have evolved diverse metabolic processes to inhabit a broad range of natural environments and can perform most chemical reactions that are thermodynamically possible to generate energy.

In anaerobic environments, such as soils, sediments, and the deep sea, some microbes employ a unique metabolic strategy to respire on the surface of iron-based minerals.² Metal-reducing bacteria possess a significant quantity of *c*-type cytochromes (*c*-Cyts) in their outer membrane (OM) and transfer electrons to the attached Fe(III) oxides during metabolism: see Figure 1(a). These bacteria produce pilus-like appendages—called bacterial nanowires, several tens of microns long—that are thought to transport electrons to Fe(III) oxides located at relatively long distances from the cell surface. Using extracellular metal oxides for respiration is essential to eliminate excess electrons generated by the oxidation of organic matter, which would otherwise inhibit energy production. The significance of this unique microbial metabolic strategy in anaerobic ecosystems has been argued extensively.²

Recent reports demonstrated that metal-reducing bacteria can also use various artificial solid materials such as graphite, gold, and indium-tin-oxide (ITO) electrodes as a terminal-electron acceptor.³ This important result highlights the potential to harvest electricity by collecting waste electrons from bacteria through the electrode in microbial fuel cells (MFCs), a type of electrochemical fuel cell.

A great deal of research has focused on identifying the OM proteins responsible for extracellular electron transfer and improving the MFC power density. However, the specific mecha-

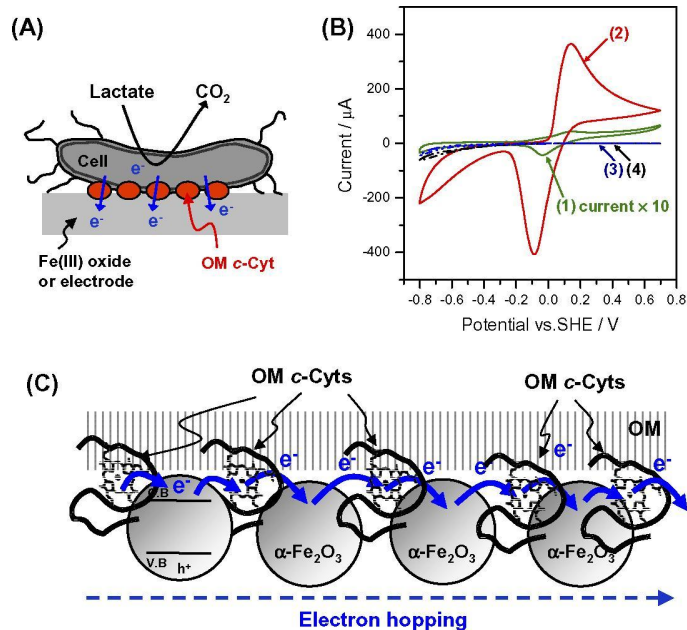


Figure 1. (a) Bacterial extracellular electron transfer mediated by outer membrane *c*-type cytochromes (OM *c*-Cyts). (b) Cyclic voltammetry of *Shewanella loihica* in the absence (trace 1) and presence (trace 2) of α -Fe₂O₃ colloids, the α -Fe₂O₃ colloidal solution lacking cells (trace 3), and the cell-free supernatant (trace 4). SHE: Standard hydrogen electrode. (c) Proposed model for the bacterial electron-transfer process mediated by α -Fe₂O₃. V.B, C.B: Valence, conduction bands.

nisms of extracellular electron transfer have not been fully evaluated in a living system. In addition, the low power density of MFCs—approximately $500\mu\text{Wcm}^{-2}$ in small-scale reactors—prevents widespread practical use.

To address these two issues we performed *in vivo* electrochemical monitoring of specific electron-transfer reactions.^{4,5} Using *Shewanella loihica* PV-4, we verified the ability of metal-

Continued on next page

reducing bacteria to self-assemble an electrically conductive bacterial network by taking advantage of the semiconductive properties of naturally abundant Fe(III) oxides: see Figure 1(a).

Shewanella possesses a large number of *c*-Cyts in the OM compartment. Because OM *c*-Cyts have a high binding affinity for Fe(III) oxides we hypothesized that the iron-oxide nanocolloids would force cells to self-assemble into an interconnected bacterial network. More importantly, we also hypothesized that—among the naturally existing crystal forms of Fe(III) oxide— α -Fe₂O₃ would serve as an effective electrical linkage because of its properties as an n-type semiconductor.

Direct evidence for the formation of an electrically conductive bacterial network was obtained by whole-cell cyclic voltammetry, a type of potentiodynamic-electrochemical measurement. We cultivated cells on the ITO electrode and confirmed that they exhibited a clear reduction-oxidation (redox) wave, having a midpoint potential (E_m) of 20mV: see Figure 1(b), trace 1. This value is in accordance with the E_m of OM *c*-Cyts isolated from Shewanella and thus demonstrates the *c*-Cyt-mediated electron-exchange process at the cell-electrode interface. Also note that when we inoculated cells in the presence of α -Fe₂O₃ colloids the peak current showed a more than 300-fold enhancement: see Figure 1(b), trace 2.

Scanning-electron-microscope images of the electrode surface revealed the formation of thick layers composed of cells and colloids, as expected. We confirmed that neither the colloids alone nor the cell-free supernatant gave a redox wave in this potential region: see Figure 1(b), traces 3 and 4, respectively. Therefore, the radical enhancement of the redox current demonstrates the formation of a long-distance electron-transfer conduit in the colloidal network, which enables many cells located a considerable distance from the electrode to participate in current generation.

Analysis of the redox properties of OM *c*-Cyts in the colloidal network revealed that an energy barrier for the electron-exchange process does not exist between the OM *c*-Cyts and the conduction band of α -Fe₂O₃. We could thus describe long-distance electron transfer on the basis of a semiconductor-mediated electron-hopping process in which the OM *c*-Cyts serve as electrical glue in the α -Fe₂O₃ colloidal network: see Figure 1(c). The proposed model of long-distance electron transfer is analogous to the operational principle of dye-sensitized photovoltaic cells, where photogenerated charges are transported over a long distance by exploiting the properties of semiconductive nanomaterials.

Semiconductive nanocolloids are abundant in natural minerals and it is not unlikely that bacteria have already taken advantage of semiconductive nanominerals in anaerobic environments to transport and share electrons in the bacterial network. We now

plan to further examine this hypothesis and its physiological implications. Moreover, we are also working toward improving the power density of MFCs to make more efficient bacterial reactors.

This work was financially supported by the Exploratory Research for Advanced Technology (ERATO) program of the Japan Science and Technology Agency (JST).

Author Information

Ryuhei Nakamura and Kazuhito Hashimoto

Department of Applied Chemistry
University of Tokyo
Tokyo, Japan

Ryuhei Nakamura is an assistant professor. He received his PhD from the Department of Engineering Science at Osaka University in 2005.

Kazuhito Hashimoto is a professor and project leader of both ERATO/JST and the Hashimoto Light Energy Conversion Project.

References

1. H. Tributsch, *On the significance of the simultaneity of electron transfer and cooperation in electrochemistry*, **J. Electroanal. Chem.** **331**, pp. 783–800, 1992.
2. D. R. Lovley, *Bug juice: harvesting electricity with microorganisms*, **Nat. Rev. Microbiol.** **4**, pp. 497–508, 2006.
3. H. J. Kim, M. S. Hyun, I. S. Chang, and B. H. Kim, *A microbial fuel cell type lactate biosensor using a metal-reducing bacterium, Shewanella putrefaciens*, **J. Microbiol. Biotechnol.** **9**, pp. 365–367, 1999.
4. R. Nakamura, F. Kai, A. Okamoto, G. J. Newton, and K. Hashimoto, *Self-constructed electrically conductive bacterial networks*, **Angew. Chem. Int'l Ed.** **44**, pp. 508–511, 2009.
5. R. Nakamura, K. Ishii, and K. Hashimoto, *Electronic absorption spectra and redox properties of c-type cytochromes in living microbes*, **Angew. Chem. Int'l Ed.** In press.