

Coordination of Nitrogen Monoxide to *c*-Type Hemes Controls Electrochemical Activity of *Shewanella loihica* PV-4

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[Introduction]

Shewanella, a dissimilatory metal-reducing bacterium, has been studied intensively due to the ability to transfer electrons to insoluble electron acceptors such as iron oxide^{1,2}. Beside electron transfer (ET) processes mediated by low molecular weight redox molecules, several reports have postulated that the ET occurs directly by redox protein complex composed of a multiheme *c*-type cytochrome (*c*-Cyt) such as OmcA and MtrC embedded into an outer membrane scaffolds¹. OmcA and MtrC have been recently isolated from the cell and the direct ET property of these proteins has been confirmed by protein film voltammetry³. However, there is no experimental evidence which prove the direct ET process mediated by multiheme *c*-Cyts in the intact cell.

To clarify the role of the outer-membrane *c*-Cyt complex in the interfacial ET between the cells and the electrode, developing the methods to monitor and control the electrochemical property of *c*-Cyt is necessary. However, for a practical experimental use of intact cells, the concentration of *c*-Cyt in a living cell is not high enough. In the course of cultivating several species of *Shewanella*, we found *Shewanella loihica* (*S. loihica*) PV-4 contains much more condensed *c*-Cyt than the others. Herein we monitored the *c*-Cyt in the outer cell membrane of *S. loihica* by exploring spectroscopic and also electrochemical methods for an intact cell. Utilizing an axial ligand-exchange reaction of *c*-type heme (Fig. 1), the cells of *S. loihica* having NO-ligated hemes were successfully achieved, and a clear change in their electrochemical activity was observed by whole-cell cyclic voltammetry.

[Material and Method]

S. loihica, PV-4 was grown aerobically at 25 °C for 40 hours in Marine-broth 20 medium. The cells containing the NO-ligated heme are prepared with NO gas bubbling in a cell medium for 5 min. The cells were then centrifuged and the solvent saturated with NO was removed. For spectroscopic and electrochemical

experiments, the cells are suspended in a defined media (DM-L), consisting of a HEPES buffer solution (pH 7.8), 10 mM of lactate and 170 mM of NaCl.

The optical absorption spectra of a cell suspension were measured in diffuse transmission (DT) mode. The cell suspension was injected into a Pyrex cell with 2 mm of optical length. The diffused transmission light was corrected by the integrating sphere located behind the Pyrex cell.

Cyclic voltammetry under an anaerobic condition was performed with a three electrode system using a platinum wire as a counter electrode, an Ag/AgCl in KCl saturated reference electrode and an indium-tin oxide (ITO) substrate working electrode placed at the bottom surface of an electrochemical cell (Fig. 2). The cell was sealed in N₂ atmosphere and filled with cell suspension of DM-L.

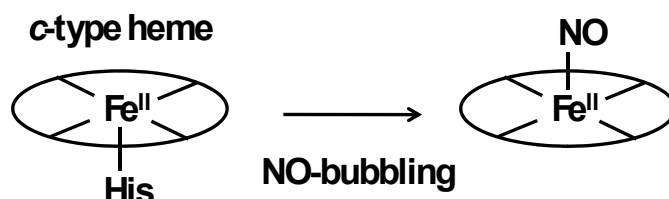


Fig. 1 NO-ligation reaction scheme of *c*-type heme

[Results and discussion]

Fig. 3 shows the DT UV-vis absorption spectrum of a suspension of *S. loihica* PV-4. The spectrum gave absorption bands that are characteristic for the reduced forms of *c*-Cyt, namely α -, β -, and γ -bands peaked at 552, 523, and 420 nm, respectively. The DT UV-vis absorption spectrum for the cell suspension after introducing NO gas showed the blue-shift in γ -band at 407 nm, which corresponds with the report on the isolated *c*-Cyt with NO-ligated heme⁴. Therefore, it was confirmed that NO was coordinated with the centered Fe of *c*-type heme (Fig. 1).

Fig. 4 shows a cyclic voltammogram of *S. loihica*. A redox wave with midpoint potential of 10 mV vs SHE was observed. For the cells having NO-ligated *c*-type heme, this redox wave completely disappeared with the shift of the rest potential from 10 mV to 400 mV. These results demonstrate that the redox wave resolved in fig. 4 is assigned to the *c*-type heme complex.

We will present details about electrochemical experiments and discuss whether outer-membrane *c*-Cyt mediates the electron directly to the ITO electrode or not.

[Reference]

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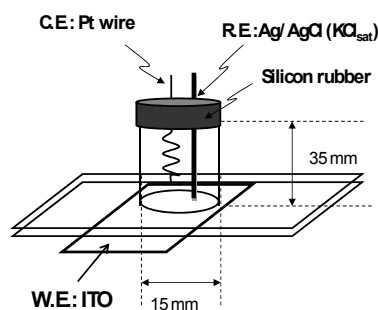


Fig. 2 Electrochemical cell with three electrode system

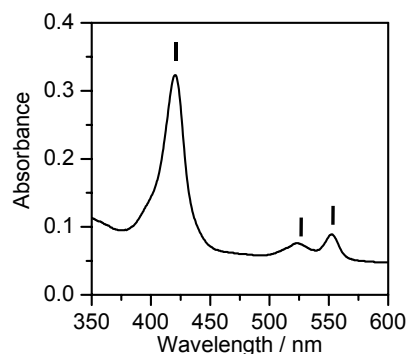


Fig. 3 DT UV-vis absorption spectrum of a cell suspension of *S. loihica*, PV-4

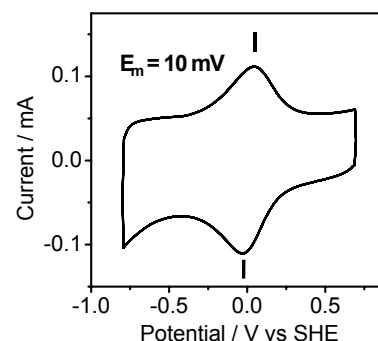


Fig. 4 Cyclic voltammogram (1 Vs⁻¹) of *S. loihica* PV-4