

The redox status of outer-membrane *c*-type cytochromes in a cell suspension of *Shewanella oneidensis* MR-1

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The process of microbial extracellular electron transfer (EET) is an important driving force of element cycling and energy exchange in epigeosphere. Although the outer-membrane *c*-type cytochromes (*c*-Cyts) of metal reducing bacteria have been well-recognized as the key enzymes in EET processes, little was known about the quantitative information of the redox status of *c*-Cyts under the non-invasive physiological conditions. The reduced and oxidized forms of *c*-Cyts in a cell suspension of a dissimilatory metal reduction bacteria *Shewanella oneidensis* MR-1 were able to be directly recorded on a UV-Vis diffuse-transmittance spectrometer, and simultaneously, the bulk redox potentials of the cell suspension were measured under the open circuit conditions. In this study, the combination of UV-Vis diffuse-transmittance spectroscopy and the electrochemistry was conducted to *in situ* investigate the redox status of *c*-Cyts and the redox potentials of the living cell suspension system. A strong correlation between *c*-Cyt_{red} concentration and open circuit voltage (OCV) was observed, implying the dominant role of *c*-Cyts redox states in controlling the extracellular electron transport capacity. The theoretical equation of the redox potentials of *c*-Cyts in the intact cells was derived based on the Nernst Equation. The fraction of *c*-Cyt_{red} and *c*-Cyt_{ox} not only reflected the electron transfer rates but also determined the formal redox potential. Effects of electron donors and electron acceptors on the fraction of *c*-Cyts was examined with results that, while the intracellular electron transfer rate was determined by electron donors, the redox potential of electron acceptors influenced the *c*-Cyts redox state and the OCV simultaneously. This study provides a new approach to investigate the redox status of outer-membrane proteins under the non-invasive physiological conditions, by which the extracellular electron transfer mechanism of dissimilatory metal reduction process can be revealed from a molecular-level view.

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