In vivo redox status of membrane-associated *c*-type cytochromes: kinetics and thermodynamics

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The *c*-type cytochromes (*c*-Cyts) in metal reducing bacteria play an important role in biomineralization processes. Although *in vitro* studies with purified proteins have demonstrated that extracellular electron transfer (EET) mediated by membrane-associated *c*-Cyts occurs very fast, *in vivo* evidence for the transient redox status of *c*-Cyts was still lacking. By employing a UV/Vis spectrometer with an integrated-sphere detector, rapid spectral changes of *c*-Cyts in living *Shewanella oneidensis* MR-1 were collected on a second and even 10 ms scale. The reaction kinetics were analysed in four stages: (i) oxidized *c*-Cyts (*c*-Cyt_{ox}) was transformed into reduced *c*-Cyts (*c*-Cyt_{red}) via intracellular electron transfer, (ii) *c*-Cyt_{red} was rapidly oxidized, (iii) *c*-Cyt_{ox} was slowly reduced to *c*-Cyt_{red}, and (iv) *c*-Cyt_{red} was regenerated. This study provides a direct molecular level observation of the *in vivo* rapid EET kinetics under non-invasive physiological conditions. To correlate the redox status of *c*-Cyts with the redox potentials, the bulk redox potentials of the cell suspension were simultaneously measured under the open circuit conditions. 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 were 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 status 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 can be revealed from a molecular-level view.

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