A diffuse-transmission spectral method for quantifying outer membrane *c*-type cytochromes in intact cells

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The outer membrane (OM) c-type cytochrome has been well-known as the key enzyme mediating extracellular electron transfer to terminal electron acceptors, resulting in the biogeochemical elemental transformation, contaminant degradation, and nutrient cycling. Although *c*-type cytochromes (*c*-Cyts) mediating metal reduction have been mainly investigated with *in vitro* purified proteins of dissimilatory metal reducing bacteria, the *in vivo* behavior of *c*-Cyts is still unclear given the difficulty in measuring the proteins of intact cells. Here, *c*-Cyts in living *Shewanella oneidensis* MR-1 was successfully quantified using diffuse-transmission UV/Vis spectroscopy due to the strong absorbance of hemes at 419 nm and 552 nm for reduced form, and 410 nm for oxidized form. The concentration of *c*-Cyts in OM *c*-Cyts of MR-1 was calibrated by horse heart c-type cytochrome according to their heme content. Because the absorbance peak at 419 nm and 410 nm usually overlapped, we established an equation to separately calculate the heme concentrations of pure reduced *c*-Cyts and oxidized *c*-Cyts. The method established was further used for the real sample with changing *c*-Cyts status, and the OD_{600}, cell numbers, DNA copies, and total proteins were examined as well. Results suggested that the most accurate and stable method for quantifying the heme content was to nominate heme concentrations by the total proteins. In addition, results implied that both the cell numbers and protein expression might influence the total heme concentrations in intact cells especially for the long-term incubation processes. Therefore, the *in situ* spectral analysis of the OM proteins in intact cells by DT-UV/Vis spectroscopy appears to be quite promising for the study of the microbial metal reduction process in a living cell system.

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