

57. A Spectroscopic Device to Monitor Respiratory Electron Transfer in Suspensions of Live Organisms

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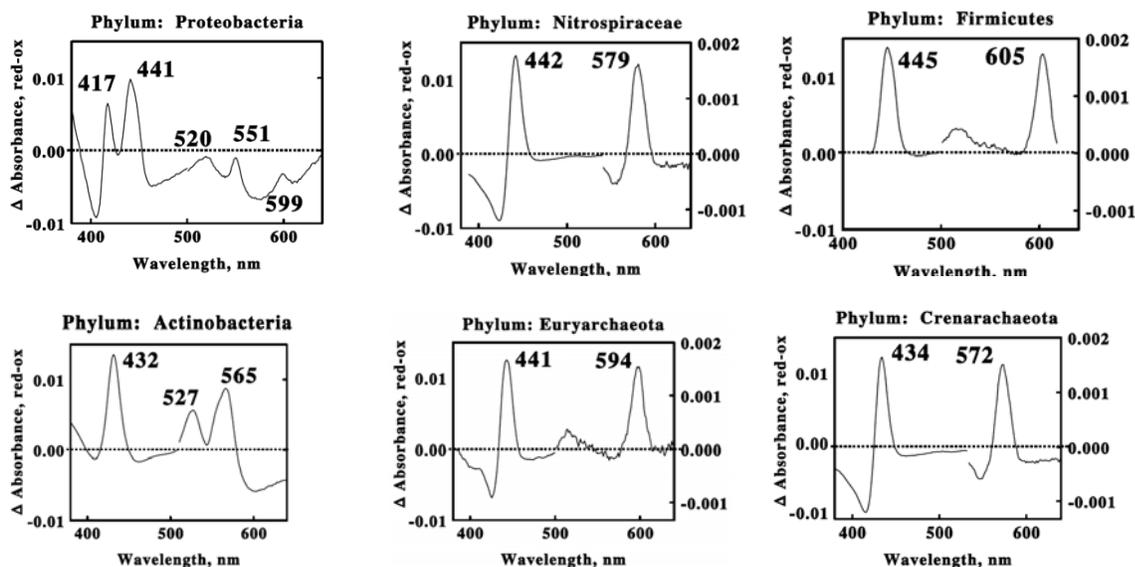
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Project Goals: The practical goal is to develop an integrating cavity absorption meter (ICAM) to directly observe respiratory electron transfer reactions in live bacteria as they exchange electrons with soluble or insoluble substrates under physiological conditions. The premise is that accurate UV-visible spectroscopy of electron transfer reactions among colored biomolecules can be conducted in highly turbid suspensions if the live bacteria are irradiated in an isotropic homogeneous field of incident measuring light. We exploited this ICAM to test the hypothesis that acidophilic bacteria in different phyla express different types of electron transfer proteins to respire on extracellular iron. We have also studied the dynamic behavior of the electron transport system at the microbe-mineral interface. The outcome of this project provides a new means to examine the extents and rates of biochemical events in situ without disrupting the complexity of the cellular environment.

An experimental beta unit of an integrating cavity absorption meter was obtained from On Line Instrument Systems (Bogart, GA), in which the cuvette is a reflecting cavity completely filled with the absorbing suspension. A simplified model of this ICAM designed to permit accurate absorbance measurements in media that scatter light is shown on the right. The observation cell is comprised of a spherical quartz cuvette fused with a quartz tube to permit access. The chamber is surrounded by a tightly packed white powder that serves to maximize diffuse reflectance of light on the exterior walls of the spherical flask. The apertures in the reflecting sphere through which the measuring light enters and the transmitted/ scattered light exits to the photomultiplier tube are positioned at a 90° angle such that the light must undergo many reflections and cell transversals before it is quantified using the detector.

Initial studies focused on respiratory electron transfer reactions in live bacteria that respire aerobically on extracellular iron at pH 1.5. Difference spectra such as those shown in Fig. 1 were obtained as soon as the suspension of cells was mixed with soluble ferrous iron. The resting absorbance spectrum of the bacterium observed under air-oxidized conditions was always regenerated from that of the Fe(II)-reduced bacterium initially observed in the presence of Fe(II).

Figure 1. Iron-reduced minus oxidized difference spectra of representative intact microorganisms from six different phyla. Spectra were obtained using the ICAM under physiological conditions at pH 1.5. Organisms: Proteobacteria, *Acidithiobacillus ferrooxidans*; Nitrospiraceae, *Leptospirillum ferriphilum*; Firmicutes, *Sulfobacillus thermosulfidooxidans*; Actinobacteria, *Ferrimicrobium acidiphilum*; Euryarchaeota, *Acidiplasma aeolicum*; and Crenarchaeota, *Acidianus brierleyi*.



In each case, the velocity of product ferric iron formation at any time point was directly proportional to the concentration of the principal reduced biomolecule(s) observed in that particular organism. Further, the integrals over time of the concentrations of the reduced biomolecules were directly proportional to the total concentrations of ferrous iron in each reaction mixture. These kinetic data obtained using whole cells were consistent with the hypothesis that the prominent reduced electron transfer biomolecule(s) observed with each organism were obligatory intermediates in the iron respiratory chains of the respective organisms. The ability to respire aerobically on extracellular iron is currently distributed among 19 genera in 6 different phyla. The different biomolecules observed in Fig. 1 were consistent with the central hypothesis that phylogenetically distinct microorganisms express different types of electron transport biomolecules to achieve respiration on extracellular iron.

There is no better means to establish physiological relevance in a metabolic function than to directly observe it as it occurs in the intact bacterium. The ability to conduct direct spectro- photometric studies under non-invasive physiological conditions represents a new and powerful approach to examine the extents and rates of biological events in situ without disrupting the complexity of the live cellular environment.

This research was supported by the Office of Biological and Environmental Research in the US Department of Energy Office of Science.