152. The Multi-Center Iron Respiratory Chain of *Acidithiobacillus ferrooxidans* Functions as an Ensemble With a Single Macroscopic Redox Potential

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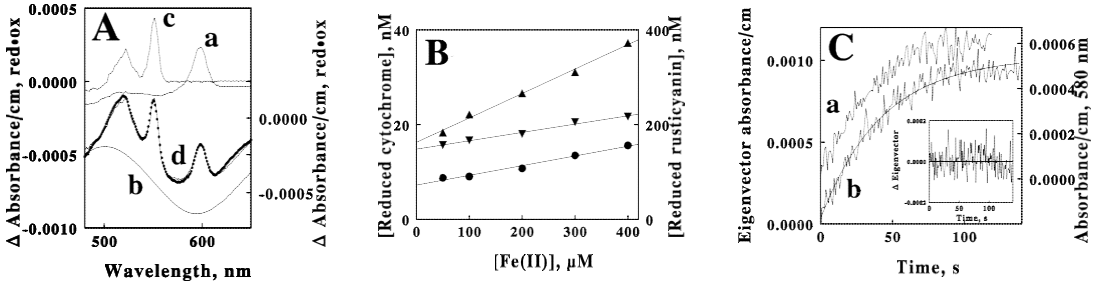
Project Goals: The practical goal is to develop an integrating cavity absorption meter (ICAM) to directly observe respiratory electron transfer reactions in live bacteria as they exchange electrons with soluble or insoluble iron under physiological conditions. The premise is that accurate UV-visible 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 will exploit 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 will also study the dynamic behavior of the electron transport system at the microbe-mineral interface. This project will provide a new means to examine the extents and rates of biochemical events *in situ* without disrupting the complexity of the live 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. Recent studies have focused on the reactions exhibited when suspensions of intact *At. ferrooxidans* were mixed with Fe(II) at pH 1.5. The electron transfer pathway for the flow of electrons from iron to molecular oxygen in *At. ferrooxidans* is hypoth- esized to consist of an initial electron transfer from extracellular ferrous ions to a cytochrome *c* located in the outer membrane of this Gram-negative organism. The periplasmic blue copper protein, rusticyanin, then transfers the electron from the cytochrome *c* in the outer membrane to a different periplasmic cytochrome *c*. The final electron transfer is from the periplasmic cytochrome *c* to the terminal oxidase, an *aa3*-type cytochrome that is located in the cytoplasmic membrane and reduces molecular oxygen. All three types of electron transport proteins were identified and readily visible in the difference spectrum represented by the *data points* in Fig. 1A. The reduced cytochrome *c* is represented by the peaks at 520 and 551 nm in the spectrum. Similarly, the reduced cytochrome *a* is represented by the peak at 598 nm in the difference spectrum shown in the figure. Finally, rusticyanin, the blue copper protein, has a broad absor- bance band at around 600 nm in the oxidized state that disappears entirely when the protein is reduced. Curves *a*, *b*, and *c* in Fig. 1A represent the difference spectra of 13 nM cytochrome *a*, 22 nM cytochrome *c*, and 370 nM rusticyanin, respectively. Curve *d* represents the sum of curves *a*, *b*, and *c*. It was evident that we could quantify the concentrations of all three types of electron transfer proteins in the intact bacterium at any concentration of Fe(II).

Figure 1. Absorbance measurements when 1.4×10^8 cells/ml of intact *At. ferrooxidans* were mixed with 300 µM Fe(II) at pH 1.5. **A**, *data points* define the difference spectrum obtained immediately after mixing. Curves representing difference spectra: *a*, cytochrome *a*; *b*, rusti- cyanin; *c*, cytochrome *c*; and *d*, sum of curves *a*, *b* and *c*. **B**, dependences of the concentrations of rusticyanin (*triangles*), cytochromes *c* (*inverted triangles*) and *a* (*circles*) on the initial concentration of Fe(II). **C**, kinetic traces for the aerobic oxidation of reduced cellular components. Curve *b* is the eigenvector absorbance from a global fit of the combined spectral changes from all three components visible in the intact bacterium; curve *a* is the absorbance change at 580 nm, representing primarily the rusticyanin alone. *Inset*, a residual plot of the global fit to the eigenvector absorbance.



Difference spectra such as that shown in Fig. 1A were obtained at different initial concentrations of Fe(II). Fig. 1B shows the dependence of the concentrations of each of the three reduced components on the concentration of ferrous iron. Despite the fact that all three electron transfer proteins exhibit quite different standard reduction potentials *in vitro*, the relative concentrations of the reduced proteins did not vary in the intact organism with the concentration of iron. That is, at sub-saturating concentrations of electrons derived from soluble iron, the three types of proteins maintained the same relative concentrations.

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). More convincing evidence for the ensemble-like behavior of the iron respiratory components came from the oxygen-dependent decay of the reduced proteins back to their resting oxidized states. Fig. 1C shows a comparison of the time courses for the oxidation of the bacter- ium. Curve *a* shows the pseudo-first order increase at 580 nm, a wavelength where the absor- bance change was primarily due to the oxidation of the rusticyanin. Curve *b* shows the identical pseudo-first order increase calculated from a multiwavelength global fit of the absorbance changes to a single exponential function of time over the range of 480 to 660 nm. It was evident that all three electron transfer proteins oxidized at the same rate, regardless of their inherent redox potential or their position in the overall respiratory chain.

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