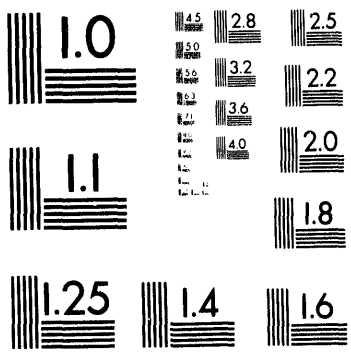
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7.1

1 of 1

DOE/ER/13339-9

MOLECULAR CHARACTERIZATION OF BACTERIAL RESPIRATION ON MINERALS

PROGRESS REPORT

June, 1992 - November, 1993

Grant No. DE-FG05-85ER13339

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DISCLAIMER

A. CURRENT SPECIFIC AIMS

The scope of work outlined in the current proposal contained three specific aims to be accomplished within three years. Accordingly, experimental progress to date on each of these aims is summarized below.

Specific Aim #1 - To continue the identification, separation, and characterization of the cellular components necessary for aerobic respiration on iron

The results of a systematic survey conducted by this laboratory on the electron transport components expressed in conspicuous quantities by acidophilic bacteria that respire aerobically on ferrous ions are summarized in the 4 enclosed reprints. The view that emerged from these studies is that different iron oxidation pathways, electron transport mechanisms, and modes of energy conservation exist in different species and genera of bacteria that respire on iron. This survey provided a basis for classifying some 130 different iron-oxidizing bacteria into one of 4 categories, based on the type of redox-active electron transport proteins expressed during aerobic respiration on reduced iron. The comparative spectroscopic analyses described in the enclosed reprints were intended to provide an overview of the most conspicuous components of the respiratory chains involved in iron oxidation as the first step in a more detailed investigation of the oxidation process in the bacteria that comprise each of the 4 categories.

Structural and functional studies on purified electron transfer components continue to focus on rusticyanin, an acid-soluble blue copper protein isolated from Thiobacillus ferrooxidans. We continue to supply purified rusticyanin to Dr. Menachem Shoham (an X-ray crystallographer at Case Western Reserve University) and Dr. Jane Dyson (a protein NMR spectroscopist) in an effort to determine the tertiery structure of the protein. In the meantime, efforts to purify and characterize other electron transfer components from cell-free extracts of T. ferrooxidans that appear to interact with the rusticyanin continue.

We have recently purified a novel red cytochrome to electrophoretic homogeneity from cell-free extracts of Leptospirillum ferrooxidans. L. ferrooxidans appears to express no rusticyanin whatsoever; instead, it produces abundant quantities of this acid-stable red cytochrome when grown on ferrous ions. The purified protein exhibited a molecular weight of 16,152 daltons by electrospray mass spectrometry. Efforts to determine the primary sequence of this protein are in progress in collaboration with Dr. Jack Shively at the City of Hope in Duarte, CA. Collaborative experiments with Dr.Russ Timkovich, University of Alabama, have revealed that the heme prosthetic group (a derivative of heme A) is covalently linked to the polypeptide by a sulfhydryl bond (a novel construction found elsewhere only in myeloperoxidase). The purified red cytochrome is redox-active with ferrous ions at acid pH, and efforts to characterize the kinetic properties of that electron-transfer reaction by stopped flow spectrophotometry are in progress.

Specific Aim #2 - To provide sufficient biochemical and behavioral information about each novel iron-oxidizing isolate to achieve a taxonomic classification

The properties of a new iron-oxidizing eubacterium, designated as strain Funis, were presented in the Geomicrobiology Journal (reprint enclosed). Strain Funis was judged to be different from other known iron-oxidizing bacteria on the bases of comparative lipid analyses, 16S rRNA sequence analyses, and cytochrome composition studies. Efforts to classify strain Funis by comparisons of its rRNA nucleotide sequence with those of published rRNA sequence signatures provided no convincing affiliations with any of the defined eubacterial divisions. More rigorous efforts to align the entire known sequence (positions 17 to 1377) of the rRNA from Funis with those in the extensive data collection are clearly warranted and will be pursued. When grown autotrophically on ferrous ions, Funis produced conspicuous levels of a novel acid-stable, acid-soluble yellow cytochrome with a distinctive absorbance peak at 579 nm in the reduced state. There were sufficient similarities between the spectral properties of this yellow cytochrome from Funis and those of the red cytochrome from L. ferrooxidans to suggest that the 2 chromophores possess common structural features. Efforts to establish whether this is true are in progress.

Current efforts focus on another new iron-oxidizing isolate, designated as strain Carla. Although strain Carla was physiologically and morphologically identical to the type strain of T. ferrooxidans, heterologous DNA hybridization studies indicated that the two organisms possess little or no DNA homology. Furthermore, strain Carla expressed a respiratory chain dominated by conspicuous quantities of a detergent-soluble c-type cytochrome not apparent in corresponding extracts from T. ferrooxidans. We have now characterized Carla-like organisms obtained from numerous sites around the world. It is clear that its occurence is widespread and that the strain must be further characterized and added to culture collections. We intend to do so.

Specific Aim #3 - To initiate an investigation of the molecular principles whereby these bacteria recognize and adhere to their insoluble inorganic substrates

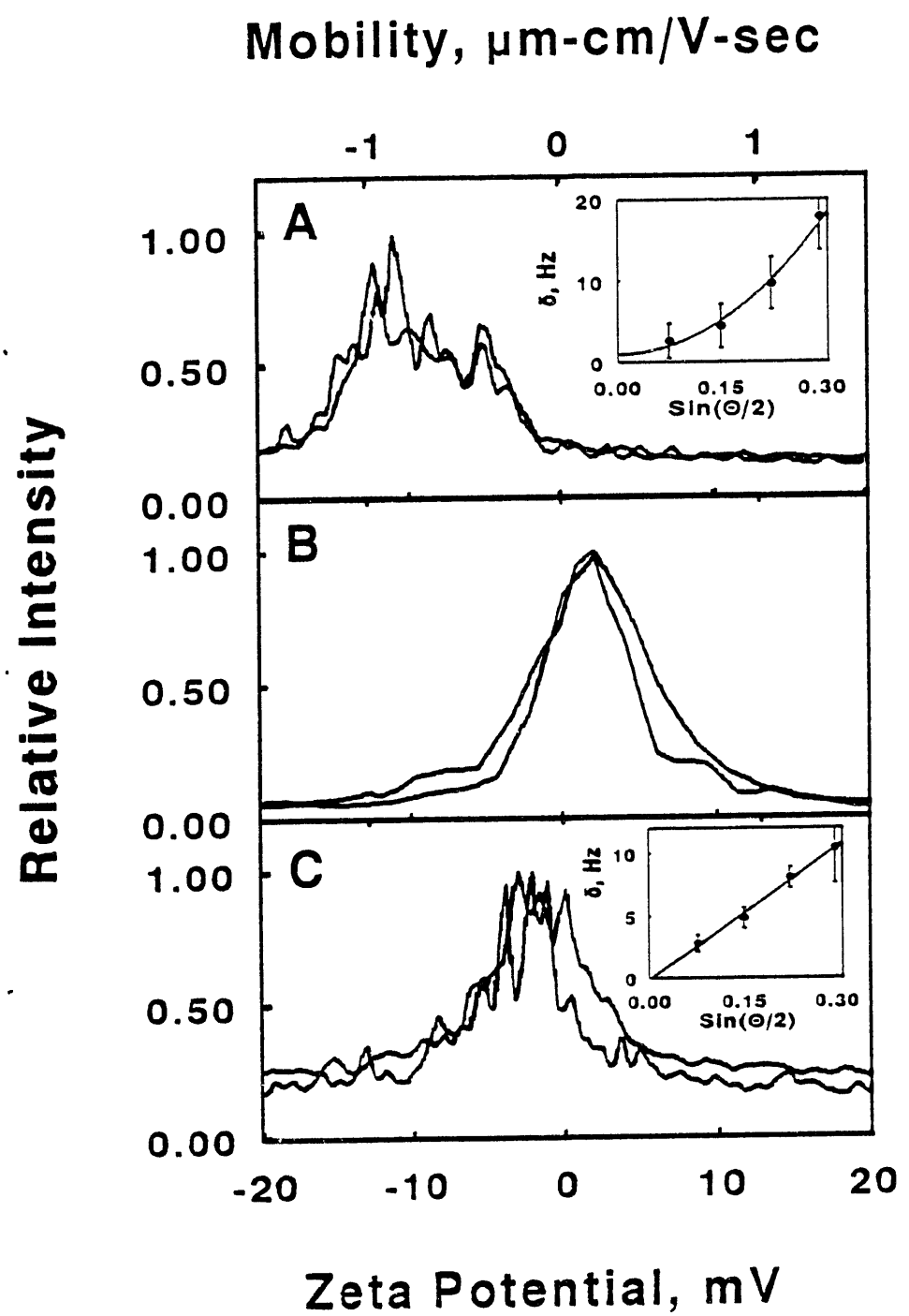
We have obtained interesting preliminary results on the interaction between T. ferrooxidans and pyrite using the DELSA 440 electrokinetic analyzer. The attached figure shows the intensity of scattered light as a function of the zeta potential (proportional to the measured electrophoretic mobility) of the light-scattering species for colloidal pyrite (panel A), bacterial cells (panel B), and a mixture of the two (panel C). The powdered, unfractionated pyrite comprised a mix of many particle sizes that was reflected in the heterogeneity apparent in the complex profile shown in panel A. The inset shows a plot of the half-width at half-height of the light-scattering profile versus the angle of the 4 independent photodetectors in the instrument.

The nonlinear dependence indicated that the finely ground pyrite was sufficiently small to be subject to considerable Brownian motion (mean diameter less than 200 microns). In the 0.01 N sulfuric acid that comprises physiological conditions for the bacterium, the pyrite was strongly negatively charged, as indicated by the zeta potential range between -10 and -20 mV. Under the same conditions the bacteria were slightly positive, as indicated in panel B. The bacteria were sufficiently large to exhibit very little Brownian motion, consequently a plot similar to that in the inset to panel A gave a linear dependence (data not shown). When the two samples were mixed and incubated for 1 hour, the pyrite- and cell-dependent profiles had disappeared and were replaced with a new, broad scattering peak that corresponded to a species with an electrophoretic mobility intermediate between those of the free components. This new peak exhibited the heterogeneity of the free pyrite but the size of the free bacteria (as indicated by the angular dependence shown in the inset of panel C). This intermediate-mobility species thus exhibited the properties one would anticipate for a complex between the bacterial cells and the particulate pyrite. We were subsequently able to observe this complex formation instrumentally using another Coulter instrument, the Multisizer IIe. The Multisizer counts colloidal objects as a function of size. Preliminary experiments on the Multisizer indicated that we can correlate the loss in bacterial counts with an apparent increase in volume of the particulate pyrite. If we can develop a quantitive assay for the binding of these acidophilic bacteria to pyrite and other mineral substrates, then we can ask some very fundamental questions regarding that interaction.

B. RENEWAL PROPOSAL

The experimental plan remains intact relative to the original proposal.

Reprints / removed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B
C
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0.50
0.00
δ, Hz
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0
0.00
0.15
0.30
Sin(Θ/2)
5
Relative Intensity
-20
-10
0
10
20
Zeta Potential, mV

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