1. Introduction

WERE FE(II) OXIDIZING PHOTOAUTOTROPHS INVOLVED IN THE DEPOSITION OF PRECAMBRIAN BANDED IRON FORMATIONS?

Banded Iron Formations (BIFs) are ancient sedimentary rocks characterized by laminations consisting of the siliceous mineral chert (SiO_2) and various Fe minerals [96]. The Fe minerals in these rocks, which by definition contain >15 wt.% Fe [80], are generally oxidized minerals, such as magnetite (Fe_3O_4) and hematite (Fe_2O_3); however formations containing reduced Fe minerals including Fe-carbonates, -sulfides or -silicates also exist [94]. Given the massive volume of these depositions, which can extend laterally hundreds to thousands of kilometers with thicknesses of hundreds of meters, BIFs are important from an economic perspective, as they provide the source for approximately 90% of the Fe ore mined globally [172].

BIFs were deposited during a period of Earth history known as the Precambrian, with the majority of these rocks having an age that ranges from ~3.8 to 1 billion years (Ga) [96]. Models to explain the formation of BIFs are both numerous and controversial and hinge on knowing when free oxygen (O_2) appeared on the Earth. Traditionally, the origin of these rocks are explained by the precipitation of iron oxide minerals that occurred when episodic upwellings

brought deep, anoxic ocean waters high in ferrous Fe [Fe(II)] concentration in contact with more oxygenated surface waters [95]. The source of this O_2 is presumed to be oxygenic photosynthetic bacteria (cyanobacteria), however, whether cyanobacteria capable of producing O_2 had evolved at the time when the most ancient of these BIFs were deposited (*e.g.,* 3.8 Ga) remains questionable [23, 148, 166]. In addition, several lines of geological evidence suggest that before approximately 2.3 Ga, the Earth's atmosphere was essentially devoid of O_2 and that reducing conditions prevailed [58, 74, 91, 145]. Thus, an open question is whether O_2 would have been present in sufficient quantities to form these ancient BIFs. Hypotheses invoking the direct oxidation of Fe(II) by UV light under anaerobic conditions have been proposed [24, 32, 62]; however, under the presumed chemical conditions of the Precambrian ocean [73], it is unlikely that this process accounts for the amount of Fe(III) required to explain these depositions [101].

A alternate hypothesis for the deposition of these formations under anoxic conditions is that they were formed as a metabolic by-product of anoxygenic phototrophic bacteria able to use Fe(II) as an electron donor for photosynthesis [67, 101, 182]. This metabolism proceeds by the reaction:

$$HCO^{3-} + 4\ Fe^{2+} + 10\ H_2O \rightarrow <CH_2O> + 4\ Fe(OH)_3 + 7\ H^+$$

and is likely to represent one of the most ancient forms of metabolism (see background and [20, 185]). While the majority of isolated Fe(II) photoautotrophs

are freshwater strains [52, 69, 70, 182], marine strains have been isolated as well [163]. Thus, ancient relatives of these bacteria likely inhabited the oceanic environments in which BIFs were deposited.

RESEARCH OBJECTIVES AND SUMMARY

The goal of this thesis has been to investigate the possibility that Fe(II) oxidizing phototrophs were involved in the deposition of BIFs. My approaches have ranged from the ecophysiological, to the geochemical, to the genetic and biochemical, with the objective being to characterize Fe(II) photoautotrophy at the molecular level in an effort to identify chemical signatures unique to this metabolism that are preserved in BIFs. The results of these investigations are described and discussed in detail in the subsequent chapters of this thesis.

In chapter two, further details concerning the search for biosignatures and their limitations, why we have chosen to focus on Fe(II)-oxidizing phototrophs and what is known about the molecular mechanism Fe(II) oxidation by *Acidithiobacillus ferrooxidans* are discussed. Portions of this chapter have been published in an article entitled “The Genetics of Geochemistry” in *Annual Review of Genetics*.

To investigate if the presence of H_2, which is reported to have been present in the Archean at concentrations of up to 300,000 ppm [170], would have inhibited Fe(II) oxidation by these phototrophs in an ancient ocean (potentially precluding a role for these organisms in BIF deposition), we investigated the effects of H_2 on the Fe(II) oxidation activity of *Rhodopseudomonas palustris* TIE-

1 (TIE-1) and *Rhodobacter* sp. SW2 (SW2). The findings of this work, described in chapter three, show that Fe(II) oxidation still proceeds under an atmosphere containing ~3 times the maximum predicted concentration of H_2 in the Archean when CO_2 is abundant. Additionally, the amount of H_2 dissolved in a 100 m photic zone of Archean ocean over an area equivalent to the Hamersley basin may have been less than 0.24 ppm. We thus conclude that H_2 would pose no barrier to Fe(II) oxidation by ancient anoxygenic phototrophs at depth in the photic zone and would not have prevented these organisms from catalyzing BIF deposition. Portions of this work will be submitted to *Geobiology*.

After demonstrating that Fe(II) photoautotrophy would have been an active metabolism in the environments where BIFs were deposited, we undertook a geochemical investigation to determine if a biologically unique Fe isotope fractionation was produced during photoautotrophic growth on Fe(II) of a pure strain, *Thiodictyon* strain F4, and two enrichment cultures. This work is the topic of chapter four and was published in an article entitled “Fe Isotope Fractionation by Fe(II)-oxidizing Photoautotrophic Bacteria” in *Geochimica et Cosmochimica Acta*. We found that these bacteria produce Fe isotope fractionations of +1.5 ± 0.2‰ where the $^{56}Fe/^{54}Fe$ ratios of the ferric precipitate metabolic products are enriched in the heavier isotope relative to aqueous ferrous iron [$Fe(II)_{(aq)}$]. This fractionation was relatively constant at early stages of the reaction and apparently independent of the Fe(II)-oxidation rates investigated. Given that our measured fractionation is similar to that measured for dissimilatory Fe(III)-reducing bacteria and abiotic oxidation of $Fe(II)_{aq}$ to ferrihydrite by molecular

oxygen, yet significantly smaller than the abiotic equilibrium fractionation between aqueous $Fe(II)_{(aq)}$and Fe(III) [$Fe(III)_{(aq)}$], we proposed two mechanistic interpretations that are consistent with our data: (1) there is an equilibrium isotope fractionation effect mediated by free, biologically produced Fe ligands common to Fe(II)-oxidizing and Fe(III)-reducing biological systems, or (2) the measured fractionation results from a kinetic isotope fractionation effect, produced during the precipitation of Fe(III) to iron oxyhydroxide, overlain by equilibrium isotope exchange between $Fe(II)_{(aq)}$ and $Fe(III)_{(aq)}$ species. Investigations performed by Andreas Kappler concurrent with this work, however, provided no evidence for the involvement of free biological ligands [89]. Thus, although these bacteria do fractionate Fe isotopes in a way that is consistent with Fe isotopic values found in Precambrian BIFs [84], we currently favor an abiotic mechanism for our measured Fe isotope fractionation. In addition, recent work with *Acidithiobacillus ferrooxidans* provides conclusive evidence that the Fe isotope fractionation associated with Fe(II)-oxidizing metabolisms is reflective of abiotic processes [8].

Upon our discovery that Fe isotopes would not be useful in identifying the activity of Fe(II)-oxidizing phototrophs in the rock record, we endeavored to define the molecular mechanism of photoautotrophic Fe(II) oxidation so that novel biosignatures for this metabolism might be identified. The results of our genetic investigations are presented in chapter five where two approaches to identify genes involved in Fe(II) photoautotrophy in TIE-1 and SW2 are described. In the portion of this chapter related to *Rhodopseudomonas palustris*

TIE-1, we describe the results of a transposon mutagenesis screen to identify mutants of TIE-1 specifically defective in Fe(II)-oxidation. The isolation of this strain and this screen are the primary work of Yongqin Jiao, a graduate student in the lab, and this work will be published as an article entitled “Isolation and Characterization of a Genetically Tractable Photoautotrophic Fe(II)-oxidizing Bacterium, *Rhodopseudomonas palustris* strain TIE-1” in *Applied and Environmental Microbiology*. I, however, was a co-author on this paper, as I developed the assay used to screen for mutants defective in Fe(II) oxidation and contributed to the interpretation of the isolated mutants. From this work, we identified two types of mutants defective in Fe(II)-oxidation and the disrupted genes of these stains are predicted to encode an integral membrane protein that appears to be part of an ABC transport system and CobS, an enzyme involved in cobalamin (vitamin B_{12}) biosynthesis. This suggests that components of the Fe(II) oxidation system of this bacterium may reside at least momentarily in the periplasm and that a protein involved in Fe(II) oxidation may require cobalamin as cofactor. In the work done on SW2, a genomic cosmid library of this genetically intractable strain was heterologously expressed in *Rhodobacter capsulatus* SB1003 (1003), a strain unable to grow photoautotrophically on Fe(II) and four cosmids that conferred Fe(II)-oxidation activity to 1003 were identified. The insert of one of these cosmids was sequenced to ~78% completion and likely gene candidates inferred from the sequence include two genes encoding predicted permeases and a gene that encodes a protein that may have redox capability. Sequence data obtained for the portion of this work related to SW2 is

presented in Appendix 1 and follow up work that I will complete subsequent to my graduation is described.

In chapter six, we present our biochemical work initiated to identify proteins upregulated or expressed uniquely under Fe(II) phototrophic growth conditions in SW2 and TIE-1. Preliminary results suggest that *c*-type cytochromes and other proteins that are exclusive or more highly expressed under Fe(II) growth conditions are present in these two strains. Whether these proteins are involved in phototrophic Fe(II)-oxidation remains to be investigated, however, precedent exists for the involvement of *c*-type cytochromes in Fe(II) oxidizing respiratory processes [6, 38, 174, 177, 189].

Conclusions, the implications of this work and perspectives for future research are the subject of Chapter 7.

Ultimately, the work done here provides a basis for understanding the molecular mechanism of photoautotrophic Fe(II)-oxidation and it is my hope that further investigations of this metabolism will lead us to new targets for biosignature development.