IRON REDUCTION BY BACTERIA: A POTENTIAL ROLE IN THE GENESIS OF BANDED IRON FORMATIONS

Kenneth H. Nealson* and Charles R. Myers**

ABSTRACT. Banded iron formations, or BIFs as they are called, represent one of the great enigmas in the Earth's geological past. The mode of formation of these ancient and widespread, layered deposits has been debated since their first discovery, and yet, in the words of Preston Cloud (1988), "Questions rise like a morning mist." The recent discovery of bacteria that can grow anaerobically by coupling carbon oxidation to the dissimilatory reduction of manganese or iron oxides (Myers and Nealson, 1988a; Lovley and Phillips, 1988) may have some relevance to our understanding of BIF formation. Such organisms provide a mechanism by which post-depositional reductive processes could lead to alternating iron-rich-iron-poor layers, simultaneously releasing isotopically light carbon (from organic carbon oxidation) for incorporation into carbonate rocks. In this paper we discuss the apparently widespread distribution of such metal reducing microbes and deal in some detail with the metal reducing and carbon oxidizing abilities of one such bacterium, *Shewanella putrefaciens* strain MR-1, presenting a model of layer formation based on these abilities. We also speculate that carbon respiration to metals was an ancient process, possibly an evolutionary precursor to oxygen respiration.

INTRODUCTION

It is difficult to imagine a more appropriate tribute to Preston Cloud than a series of papers dealing with various aspects of biogeochemistry, written by his admirers and the people he has so vastly affected. The interdisciplinary approach, which is so typical of Cloud's thinking and work over the past years, is now emerging, and appropriately so, as a proper and even necessary way to look at complex systems. We owe a debt of gratitude to Pres Cloud for his thinking, teaching, and sharing of knowledge—perhaps this volume is a small payment on the debt. In this report, we will present data dealing with the physiology of bacterial metal reduction, followed by a speculative discussion of the possible role of iron reduction in the genesis of banded iron formations, and, finally, we will address the question of the evolution of metabolic pathways and the importance of the iron reduction pathway. Some of these ideas were "born" during discussions with Dr. Cloud at a Dahlem Conference nearly a decade ago—the hypothesis was present, but the microbial mechanism was lacking. In the intervening years, organisms have been found and characterized that allow the construction of a "straw man"
hypothesis. We hope the hypothesis is viewed as such—a few facts and ideas in search of their place in the overall picture.

Many different layered metal deposits are encountered on the Earth, ranging from the vast Precambrian banded iron formations (BIFs) to modern day iron-manganese crusts and nodules found on sediments of lakes and oceans. Associated with layering of these deposits is usually a cyclic change in iron concentration from layer to layer. In attempts to reconstruct and explain BIFs, it has been customary to use depositional models, in which cyclic changes in the deposition of iron occur (see Holland and Schidlowsky, 1982, and references therein). While such models may not completely explain the myriad of questions that arise concerning the BIF origins (see Cloud, 1973, 1974, 1988), they are perhaps the best available, given the facts at hand.

The reconstruction of ancient ecosystems and processes is, of course, a tricky business (Cloud, 1974, 1988), one that requires an amalgamation of the geological and fossil records with the physiological, structural, and metabolic properties of extant organisms. With this in mind, we present some relatively new findings regarding organisms that live anaerobically by the dissimilation of iron oxides, suggest a possible role for such metabolic activity in the formation of BIFs, and speculate as to the possible importance of this pathway in the evolution of aerobic respiration.

IRON REDUCTION: DIAGENETIC FORMATION OF IRON LAYERS

About 20 yrs ago, it was suggested, but considered unlikely, that diagenetic, reductive activities could be a factor in the cyclic banding seen in BIFs (Perry and others, 1973). About 10 yrs later, it was again suggested that iron reduction should at least be considered with regard to BIF layering (that the layers of low iron content might be due to removal of iron by bacteria at the expense of carbon oxidation) (Nealson, 1982). At the time, however, there were no organisms known in which iron reduction was coupled to carbon oxidation and/or growth, and in the absence of a mechanism or a causative organism, this hypothesis received little support.

More recently, a similar suggestion was again put forward to explain the iron banding in Precambrian banded iron formations (Baur and others, 1985; Walker, 1984). This hypothesis resulted from a consideration of isotopic fractionation results obtained from studies of $^{13}$C/$^{12}$C ratios of the carbonate minerals contained in banded iron formations (Becker and Clayton, 1972; Baur and others, 1985), which revealed an anomalously light carbon content of the carbonate rocks associated with iron depletion. The hypothesis erected to explain these data was that the carbonate minerals were isotopically light because they were formed from CO$_2$ derived from bacterial respiration during the mineralization of organic carbon. The most likely candidate for such activity would have been respiratory bacteria capable of coupling the oxidation of organic matter to the reduction of Fe(III). According to this hypothesis,
iron would have been originally deposited as ferric iron, probably the result of interaction with molecular oxygen produced by oxygenic photosynthesis. Along with the ferric iron would be deposited the remains of the algae, composed of isotopically light carbon. Under the ensuing anaerobic conditions, diagenesis would occur due to the activity of iron reducing respiratory bacteria, resulting in the reduction of iron, and the production of isotopically light CO₂. Such a model would account for the banding of iron as well as the associated isotopic banding observed in the carbonate minerals (Baur and others, 1985). At the time of this work, although there was good geochemical evidence that iron was used as an electron acceptor for the oxidation of organic carbon in modern anaerobic ecosystems, there were still no organisms known that could couple the oxidation of organic matter to the reduction of iron in an energetic fashion.

Clearly, two pieces of microbiological information would be very helpful in assessing the likelihood that such processes might have occurred. First, one would like to have proof of a physiological or biochemical mechanism by which iron reduction can be driven at the expense of carbon oxidation, and, second, assuming that such a process is found, one would like to have some notion of its “evolutionary age” (whether the process is an ancient or modern one). With regard to the first question, considerable evidence has accumulated during the past few years and is discussed below. As to the second question, it now appears that it will be possible to gain an answer, although there are as yet no data available.

**ISOLATION AND CHARACTERIZATION OF IRON REDUCERS**

During the past few years two organisms were isolated and characterized that have the capacity to grow anaerobically using manganese or iron oxides as electron acceptors. Lovley and Phillips (1988) isolated an as yet unidentified obligately anaerobic organism (called GS-15) from anaerobic mud of the Potomac River, where iron reduction is a major process. GS-15 utilizes several different carbon compounds for energy, converting them to CO₂, while using Fe(III) as the electron acceptor. Under appropriate anoxic conditions, GS-15 reduces copious quantities of iron oxides and is capable of converting iron oxides to magnetite (Lovley and others, 1987). GS-15 also apparently utilizes either Mn(IV) or NO₃ as an electron acceptor for carbon oxidation and growth (table 1).

Myers and Nealson (1988a) isolated and identified as Alteromonas putrefaciens (now renamed Shewanella putrefaciens (MacDonnell and Colwell, 1985; International Journal of Systematic Bacteriology, 1986) an organism capable of anaerobic growth using either Mn(IV) or Fe(III) as electron acceptors. This bacterium, called MR-1, is a facultative anaerobe, capable of reduction of many other electron acceptors (table 1); it may well be the most versatelle respiratory organism ever reported. MR-1 was isolated by using an enrichment culture procedure that utilizes
non-fermentable carbon compounds as a source of energy. Using this approach, it is a routine matter to isolate iron reducers, and they are found to span a wide range of bacterial groups, including both Gram positives to Gram negatives and facultative anaerobes to strict anaerobes. It is clear from table 1 that the ability to couple iron reduction to growth is not at all uncommon. If so, why was the process not previously detected? The most probable explanation is that rich carbon sources were used for the isolations, and that the metal reducers were simply outcompeted by fermentative bacteria that altered the pH conditions or outgrew them. The enrichment culture approach avoids these problems. While the remainder of the discussion in this report will focus on only one organism, MR-1, it should be remembered that the ability to couple iron reduction to carbon oxidation is apparently phylogenetically widely distributed and that such organisms display a great deal of ecological versatility.

PHYSIOLOGY AND METABOLISM OF MR-1

MR-1 has been shown to couple the oxidation of organic matter such as lactate or acetate to the reduction of iron and to generate energy via Fe(III) dependent respiration-linked proton translocation (Myers and Nealon, 1989, 1990). A similar result with regard to iron-dependent proton translocation was reported for a manetotactic bacterium (Short and Blakemore, 1986). While no iron respiratory proteins have

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** GS-15 (Lovley and Phillips, 1988) and MR-1 (Myers and Nealon, 1988a) have been previously described. All other organisms were isolated from the water column of the Black Sea in June 1988 by Myers and Nealon (unpublished).

+++ = positive for growth/reduction; -- = negative for growth/reduction.
yet been purified, the physiological data indicate that the organisms directly interact with ferric iron and are capable of energy generation coupled to iron reduction. When grown with oxygen as the electron acceptor, proton translocation was observed only with oxygen, but when grown with other electron acceptors (for example, nitrate, fumarate, et cetera) proton pumping to many different electron acceptors was demonstrated (Myers and Nealson, 1989;1990).

Consistent with its iron reducing-energy generating ability is the observation that MR-1 is a non-fermentative, obligatorily respiratory organism, which dies rapidly in the absence of an electron acceptor. Considering this dependence on respiratory energy generation, it is perhaps not surprising to find that MR-1 has the ability to utilize many different electron acceptors (table 1).

With regard to carbon metabolism, MR-1 grows on a variety of organic compounds, many of which are compounds commonly found in anaerobic ecosystems, including lactate, pyruvate, acetate, and ethanol (Myers and Nealson, 1990). Figure 1 shows the anaerobic growth of MR-1 using lactate as the sole source of carbon and energy and several different electron acceptors; no growth was observed when succinate was used as the sole source of carbon and energy. The production of reduced iron during growth on various carbon and energy sources is

![Graph showing anaerobic growth of MR-1](image)

Fig. 1. Anaerobic growth of MR-1 with lactate as the sole source of carbon and energy. The experiments were conducted at room temperature (about 25°C) in a liquid defined medium, pH 7.4 (Myers and Nealson, 1988a), and an atmosphere of 100 percent nitrogen. The electron acceptors utilized are indicated on the figure. Good growth was obtained with TMAO (trimethyl amine oxide), nitrate, and ferric iron, but no growth was observed if the electron acceptor was omitted.
shown in figure 2. Iron reduction is most rapid on lactate, on which the best growth is obtained, and lowest on succinate, on which growth does not occur. The carbon sources that can be used as sources of carbon and energy and as sources of carbon are shown in table 2. MR-1 uses only a few carbon compounds for energy, but, given an energy source, it is capable of utilizing many different sources of carbon for growth. While the details of carbon oxidation (the nature and stoichiometry of the carbon products as related to iron reduction) by MR-1 are still being elucidated, it is already clear that these organic acids are converted primarily to CO₂, with some of the carbon being incorporated into biomass.

In summary, MR-1 and, presumably, many other iron respiratory iron reducers are clearly capable of coupling their growth and metabolism to the oxidation of organic carbon, oxidizing the organic matter to CO₂. These organisms provide us with a physiological and biochemical

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**Fig. 2.** Iron reduction by MR-1 with various carbon sources. The experiments were conducted at room temperature (about 23°C) in a liquid defined medium, pH 7.4 (Myers and Nealson, 1988a) and an atmosphere of 100 percent N₂, with 4mM amorphous ferric oxyhydroxide (prepared according to Lovley and Phillips, 1986) as the sole electron acceptor. The medium was supplemented with various carbon compounds as indicated. Fe(II) reduction was monitored by measuring the accumulation of Fe(II) over time by the ferrozine extraction procedure (Lovley and Phillips, 1986; Myers and Nealson, 1988b). The amount of Fe(II) reduction shown is the net amount of reduction above any that may have occurred in the sterile cell-free control with the same carbon source(s).
Table 2

Carbon and energy metabolism of MR-1

<table>
<thead>
<tr>
<th>I. Compounds that can be used as sole sources of carbon and energy:</th>
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<td>Organic Acids</td>
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<td>lactate</td>
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<th>II. Compounds that stimulate the growth of MR-1 on acetate:</th>
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<td>TCA Compounds</td>
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*Stimulates aerobic growth only.
**Stimulates anaerobic (100 percent N₂) growth only.
***Stimulates anaerobic (100 percent N₂) growth only. Inhibits aerobic growth on acetate plus Casamino acids. Formate can serve anaerobically as a source of energy for MR-1 but not as a sole source of carbon.

Role of iron reduction in layer formation

A scenario for formation of alternating iron-rich and iron-poor layers, utilizing the respiratory abilities of an MR-1 like organism is shown in figure 3. For Precambrian banded iron formations it is hypothesized that iron deposition occurred seasonally as a result of oxygenic photosynthesis during the growing season. Since ferrous iron should have been abundant in the world's oceans at this time, virtually all the oxygen produced would have led to production and precipitation of iron oxides, probably in the form of FeOOH or Fe(OH)₃ (Cloud, 1974, 1988). Although the model shows iron precipitation due to oxidation by molecular oxygen, the product of oxygenic photosynthesis, this is not a critical feature of the model: the important thing is that ferric iron should be deposited. For example, Hartman (1984) proposed that anaerobic photosynthetic bacteria might utilize iron as an electron donor, leading to the deposition of ferric iron under anaerobic conditions. It is essential to our thesis, however, that the carbon deposited as organic matter be of autotrophic (light carbon) origin.

Organic carbon that accumulated on the surface of the oxidized iron would then provide the material and conditions for microbial iron reduction at the expense of carbon oxidation. Since these two processes are metabolically coupled (Myers and Nealson, 1988a), the extent of
I. OXIDATION OF FERROUS IRON

\[ \text{Fe}^{2+} \rightarrow \text{FeOOH} \quad \text{Fe}_2 \text{O}_3 \quad \text{Fe}(	ext{OH})_3 \]

\[ \text{CO}_2 \quad \left( \text{Photosynthesis} \right) \]

\[ \left( \text{CH}_2 \text{O} \right)_n \quad \text{(organic carbon*)} \]

II. SEDIMENTATION OF IRON OXIDES/ORGANIC CARBON

\[
\begin{array}{c}
\text{FeOOH} \quad \text{Fe}_2 \text{O}_3 \quad \text{Fe}(	ext{OH})_3 \\
\text{organic carbon*}
\end{array}
\]

III. IRON REDUCTION/CARBON OXIDATION

\[ \text{Fe}^{3+} \rightarrow \text{Fe}^{2+} \]

\[ \text{CO}_2 \rightarrow \text{Ca}^+ \text{CO}_3 \]

IV. COMPLETION OF ONE CARBON OXIDATION/IRON REDUCTION CYCLE

Fig. 3. Model of Iron Layer Formation. This scenario is based on many previously proposed schemes and incorporates the activity of MR-1 like organisms in one of the steps. We assume that the first step is the oxidation of ferrous to ferric iron (step I) by oxygen that is produced by oxygenic photosynthetic algae, blooming in large basins into which iron-rich water has been upwelled. This oxidized iron then precipitates to the bottom, forming an iron-rich bottom sediment (step II). As a second part of step II, organic matter precipitates to the bottom. This might occur simultaneously with the iron, or later, after the crash of algal bloom. In step III, iron reduction by MR-1 like organisms results in the net conversion of organic matter to CO$_3$ and the reduction of ferric to ferrous iron. The CO$_3$ (*) = isotopically light) is fixed into carbonate rocks, while the ferrous iron either diffuses away, interacts with other iron species, forming magnetite, or both. When the reduction is complete (all the metabolizable carbon is oxidized), the cycle is over and ready for another input of iron-rich sediment with the next algal bloom.
Iron reduction would, of course, depend on the amount of organic carbon added to the sediment. However, assuming that the carbon added was less (in reducing equivalents) than the iron that was originally laid down, then an iron-poor layer would be produced on top of an iron-rich one. Such processes on a yearly basis (or any cyclic period) would lead to a regular banded deposit, with the thickness of the iron-poor band dependent on the amount of organic carbon deposited.

According to this scenario, if diagenetic events were responsible for iron depletion, isotopically light carbonate minerals should accumulate (Baur and others, 1985). Furthermore, the involvement of iron in the oxidation of organic carbon would yield iron in the reduced state, either as ferrous iron, which could migrate out of the environment, or possibly accumulate as the mixed valence iron mineral magnetite (Bell, Mills, and Herman, 1987: Lovley and others, 1987).

**EVOLUTION OF RESPIRATORY PATHWAYS**

The discussion of ancient sedimentary environments and the role of iron reduction in banded iron formations brings up an interesting point regarding the evolution of metabolism. During the time when the deposition of banded iron formations began, the evolution of respiration to oxygen almost certainly had not occurred. Oxygen had not yet accumulated to any significant degree on the surface of the Earth, and ferrous iron and other inorganic ions would have acted as oxygen sinks, outcompeting any oxygen respiratory systems. However, electron transfer systems containing iron-sulfur centers and ferredoxin-like proteins, which would be ideal candidates for interacting with Fe(III) as an electron acceptor, had almost certainly evolved, as evidenced by their widespread distribution among the Archaeabacteria (George and others, 1985). Given the widespread deposition of ferric iron as a consequence of production via oxygen photosystem II, respiration to ferric iron would be a logical evolutionary consequence. Under anaerobic conditions, ferric iron is soluble enough to interact well with cells, and the presence of both ferredoxins (with their iron-sulfur centers) and other iron-containing electron transport systems would make interaction with iron a rather simple evolutionary step. During the hundreds of millions of years during which ferric iron was deposited and sedimentary organic matter began to accumulate, it seems quite possible that a simple respiratory chain to ferric iron could have developed, and even that this perhaps was a precursor to more complex chains we associate with extant respiratory organisms.

While this is by no means a novel thought, it is mentioned here because it is now clear that by the analysis of the genes responsible for iron reduction from extant organisms, one may get some clues as to their genetic lineages. Are iron reductases precursors, or descendents of,
other reductases? Can the time of origin of this pathway be specified? Using modern molecular genetics techniques, it should be possible to isolate the iron reductase genes, sequence them, and by comparative sequence analysis obtain some of the answers to the above questions. Such information should allow one to assess the evolutionary "age" of iron reduction: is it an ancient process; could it have been active in the Precambrian and played a role in the formation of banded iron formations?

We end where we began this article, with a tribute and a thank you to Preston Cloud, a man whose interests are so broad and encompassing that he can write books on the origin and geology of the Earth while convincing microbiologists to isolate and study new organisms and (even to isolate and sequence specific genes)—ever aware that knowledge of the present may shed light on the past, and vice versa.

ACKNOWLEDGMENTS

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STRATIGRAPHIC SHIFTS IN CARBON ISOTOPES FROM PROTEROZOIC STROMATOLITIC CARBONATES (MAURITANIA): INFLUENCES OF PRIMARY MINERALOGY AND DIAGENESIS

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ABSTRACT. In the Atar Group of Mauritania (about 900 Ma), a shift from positive (+0.3 to +2.8 permil) to variably negative (−0.2 to −3.3 permil) carbon isotope values of carbonate carbon occurs between Formation 1-5 with its Conophyton-Jaculiphyton biostromes and Formation 1-6 in which the stromatolites display tussocky microstructure.

1-5 stromatolites have filmy microstructures with micrite laminae separated by radiaxial calcite cement. The primary marine precipitates have been neomorphosed, as indicated by patchy luminescence and variably negative oxygen isotopes. Textures indicate a primary Mg-calcite mineralogy. Consistently low Sr values indicate a high water-rock ratio during neomorphism whose timing apparently overlapped with formation of sparry calcite filling small fenestrae. Growth of saddle dolomite and coarse calcite spar followed. General consistency of carbon isotope values indicates these diagenetic changes occurred during a Proterozoic phase of burial, probably to at least 3 km depth. Extensive replacive dolomitization could be a much later event however.

Stromatolitic carbonates from Formations 1-6 and 1-7 exhibit alternating micritic and detrital laminae and radiating upwardly-growing tussocks. Pseudo-hexagonal radiating gypsum pseudomorphs occur at one level. Styles of textural preservation indicate a primary aragonite composition. A strong covariation of increasing Fe and Mn with decreasing δ18O, δ13C, and Sr characterizes the geochemistry. Uniform composition of sub-samples from hand specimens indicates an open chemical system during neomorphism to calcite. The distinct geochemical patterns compared with 1-5 indicate that neomorphism was delayed until later Proterozoic uplift related to the Pan-African events allowed penetration of isotopically-depleted meteoric water from the Mauritanide mountain chain. The exceptionally low Sr isotope values reported for Formation 1-6 may well reflect input of light Sr from leaching of igneous rocks during this meteoric alteration.

Extrapolation of data points along chemical trends toward less altered values allows the carbon isotope profile to be corrected for diagenesis. When this is done the 1-5/1-6 boundary is seen to mark only a small change in δ13C, and there is no evidence of variation

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