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Phototrophic Fe(II) oxidation in an atmosphere of H_2: implications for Archean banded iron formations

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ABSTRACT

The effect of hydrogen on the rate of phototrophic Fe(II) oxidation by two species of purple bacteria was measured at two different bicarbonate concentrations. Hydrogen slowed Fe(II) oxidation to varying degrees depending on the bicarbonate concentration, but even the slowest rate of Fe(II) oxidation remained on the same order of magnitude as that estimated to have been necessary to deposit the Hamersley banded iron formations. Given the hydrogen and bicarbonate concentrations inferred for the Archean, our data suggest that Fe(II) phototrophy could have been a viable process at this time.

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INTRODUCTION

Reconstructing the evolution of metabolism is a stimulating endeavor, yet riddled with challenges. Various approaches have been used in this pursuit, all of which have their limitations. For example, molecular biomarkers provide a link to specific time intervals in Earth history, but are only as valid as they are functionally linked to the metabolisms they are thought to represent (Rashby *et al.*, 2007). Stable isotopes can be a powerful means to track the rise of different physiological groups, provided the metabolisms in question utilize an element for which multiple stable isotopes exist and produce fractionations that are straightforward to interpret after diagenesis (Johnston *et al.*, 2005). Genomics may also be helpful in inferring the evolution of different groups of organisms with particular metabolic functions, or the evolutionary history of specific enzymes, yet this approach operates on a relative temporal scale and may be confounded by horizontal gene transfer (House, 2007). Ultimately, contributions from all of these, and other, approaches will be necessary to paint a coherent picture of the metabolic evolution of early life.

In this note, we introduce a complimentary approach, 'palaeo-ecophysiology', to assess whether a particular metabolism might have been active during a given interval of Earth history. Ecophysiological approaches are commonly used in the field of microbial ecology, but to date, have been applied only sparingly in geobiology. Modern ecophysiological studies attempt to assess the likelihood of the occurrence of a particular metabolism in a given environment by integrating what is known about particular variables in that environment with information on how these variables affect the metabolic processes of interest (Kopps & Pommerening-Roser, 2001). 'Palaeo-ecophysiology' attempts the same, only it depends on limited geochemical data to infer the variables of interest, and assumes that modern microbes follow the same physiological rules as their ancient counterparts. These constraints aside, a palaeoecological approach has the potential to constrain, at least theoretically, the relevance of particular metabolisms to specific periods in Earth history. As an example, we consider the case of phototrophic Fe(II) oxidation in the Archean.

It has been suggested that anoxygenic photoautotrophs able to use ferrous iron [Fe(II)] as an electron donor for photosynthesis were involved in the deposition of banded iron formations (BIFs) that formed prior to the rise of O_2 (Hartman, 1984; Widdel *et al.*, 1993; Konhauser *et al.*, 2002; Kopp *et al.*, 2005). This model assumes that these bacteria used Fe(II) as an electron donor for photosynthesis. However, many anoxygenic phototrophs, including those able to oxidize Fe(II), are capable of using a variety of electron donors for

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photosynthetic growth. One such donor that is broadly used by diverse phototrophic bacteria is hydrogen gas (H_2) (White, 1999).

The atmosphere of the early Earth is thought to have contained between 1000 and 300 000 p.p.m. of H_2 as a result of volcanic emissions and atmospheric photochemical reactions (Kasting, 1993; Catling *et al.*, 2001; Tian *et al.*, 2005). These quantities of H_2 are sufficient to support H_2-based photoautotrophy, and it is therefore relevant to determine whether such quantities could have interfered with phototrophic Fe(II) oxidation. Coupling an understanding of the conditions under which phototrophic Fe(II) oxidation proceeds with biogeochemical/stratigraphic reconstructions of the ancient environment can help refine models that consider the role of these phototrophs in BIF deposition at different times in Earth history.

Fe(II) oxidation still proceeds at significant rates in the presence of H_2

To investigate the effects of the presence of H_2 on phototrophic Fe(II) oxidation we studied *Rhodopseudomonas palustris* strain TIE-1 (Jiao *et al.*, 2005) and *Rhodobacter* species strain SW2 (Widdel *et al.*, 1993; Croal *et al.*, 2007). Rates of Fe(II) oxidation in cell suspensions of these strains were measured under conditions where the concentrations of Fe(II), bicarbonate and H_2 were comparable to those thought to be relevant for the Archean environment. We did not attempt to mimic other relevant aspects of the Archean ocean (e.g. silica content), as the experiments reported here grew out of work done in a growth medium routinely used for the cultivation of anoxygenic phototrophs. Specifically, the initial Fe(II) concentration of ~0.5 mM was within the upper range of 0.054–0.54 mM predicted by Ewers (1983) and Holland (1973), the bicarbonate concentration of 20 mM was on the same order as the 70 mM predicted for an Archean ocean and an order of magnitude higher than the present-day concentration of 2 mM (Grotzinger & Kasting, 1993; Ohmoto *et al.*, 2004) and the H_2 concentration of 800 000 p.p.m. was also on the same order as the recently proposed concentration in the prebiotic early atmosphere of 300 000 p.p.m. (Tian *et al.*, 2005). Recognizing that other organisms (such as methanogens) can consume H_2, that methanogens are thought to be an ancient life form present in significant numbers early in Earth history (Ferry & House, 2006), and that CH_4 has been estimated to be as much as 10^2–10^3 p.p.m. in the Archean atmosphere, the amount of H_2 used in our studies is likely an over-estimate of what ancient phototrophs encountered.

Suspensions of TIE-1 and SW2 cells cultured phototrophically on H_2 and harvested in early exponential phase (OD_{600} ~0.15–0.18) by centrifugation (RCF 12320 for 20 min) were prepared for the Fe(II) oxidation assay in a COY anaerobic chamber. Pellets were washed once with an equal volume of 50 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) buffer containing 20 mM NaCl at pH 7 (assay buffer) and resuspended in 1 mL of assay buffer containing 0.5 mM $FeCl_2 \cdot H_2O$ and either 1 mM or 20 mM $NaHCO_3$ to a final OD600 of 1.0 in 12 mL sealed serum bottles. Resuspending the cells to the same final OD600 ensured that the assays were normalized to cell number, as verified by cell counts using a Petroff–Hauser counting chamber. The headspace of the assay bottles was then exchanged with either N_2/CO_2 (80 : 20 [v/v]) or H_2/CO_2 (80 : 20 [v/v]) and assay bottles were shaken vigorously every 20 min during the assay to equilibrate the gas and liquid phases. Cell suspensions of TIE-1 and SW2 were incubated at 30 °C and 16 °C, respectively, 30 cm from a 34-W tungsten incandescent light bulb and Fe(II) concentrations in the cell suspensions were measured by the ferrozine assay as described previously (Stookey, 1970; Croal *et al.*, 2007).

When the bicarbonate concentration was low, in the absence of H_2, the initial rates of Fe(II) oxidation for strains TIE-1 and SW2 were ~0.07 mM h^{-1} and ~0.15 mM h^{-1}, respectively (Fig. 1, Table 1). Under the same low bicarbonate conditions, in the presence of H_2, the rate of Fe(II) oxidation by strain TIE-1 decreased by ~43% compared to its rate in the absence of H_2. Fe(II) oxidation by strain SW2 was even more dramatically affected: during the first 5 h of the assay, the rate of Fe(II) oxidation by strain SW2 in the presence of H_2 decreased by ~80% as compared to the absence of H_2. Furthermore, after 10 h, only ~22% of the total Fe(II) added initially was oxidized.

In contrast, in the presence of high concentrations of bicarbonate, while the rates of Fe(II) oxidation decreased for both

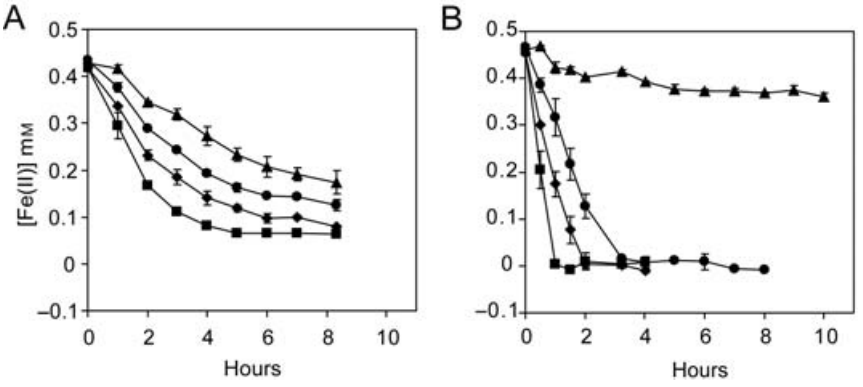

Fig. 1 The effect of H_2 on the Fe(II) oxidation activity of strains TIE-1 (A) and SW2 (B) varies depending on the concentration of $NaHCO_3$. ▲: H_2 + 1 mM $NaHCO_3$; ●: N_2 + 1 mM $NaHCO_3$; ◆: H_2 + 20 mM $NaHCO_3$; ■: N_2 + 20 mM $NaHCO_3$. Data are representative of two independent experiments. Error bars represent the error on duplicate cell suspension assays for strain TIE-1 and triplicate assays for strain SW2.

Table 1 Comparison of the Fe(II) oxidation rates for strains TIE-1 and SW2 under low bicarbonate (1 mM) and high bicarbonate (20 mM) concentrations in the presence or absence of H_2. Rates were calculated using the first three time points for all conditions in Fig. 1 except for strain SW2 under low bicarbonate concentrations in the presence of H_2; here the first five time points were considered

	Low $NaHCO_3$		High $NaHCO_3$	
	$+H_2$	$-H_2$	$+H_2$	$-H_2$
Strain	(mM Fe(II) oxidized h^{-1})		(mM Fe(II) oxidized h^{-1})	
TIE-1	0.04	0.07	0.09	0.13
SW2	0.03	0.15	0.28	0.46

strains in the presence of H_2, their decrease was less dramatic as compared to the results in the presence of low bicarbonate. For strain TIE-1, the initial rate of Fe(II) oxidation decreased ~31% as compared to that in the absence of H_2, whereas for strain SW2, the initial rate of Fe(II) oxidation decreased ~39% (Fig. 1A,B, Table 1). Under these conditions in the absence of H_2, SW2 oxidized all of the Fe(II) within 2 h, whereas TIE-1 took 8 h, revealing a significant difference in their Fe(II) oxidation kinetics (Fig. 1A,B). This difference is consistent with the fact that these organisms have different enzymatic systems that control this process (Croal *et al.*, 2007; Jiao & Newman, 2007). In all cases, however, the observed decrease in the rate of Fe(II) oxidation in the presence of H_2 was not due to Fe(III) reduction coupled to H_2 oxidation: cell suspensions to which 1 mM bicarbonate and 0.5 mM ferric (hydr)oxide (synthesized according to the method of Schwertmann & Cornell (1991)) were added did not produce detectable amounts of Fe(II) in the presence or absence of H_2 as measured by the ferrozine assay (data not shown). The underlying cellular mechanism(s) whereby H_2 inhibits Fe(II) oxidation (e.g. competition for electron acceptors in the electron transport chain, affects on production of key catalytic enzymes, etc.) await elucidation. Given that the extent of inhibition is sensitive to the bicarbonate concentration, we favour the hypothesis that when the terminal electron acceptor (e.g. CO_2) is low, H_2 may kinetically outcompete Fe(II) for oxidation by the electron transport chain.

Together, these results indicate that phototrophic Fe(II) oxidation may be affected by H_2 in modern environments where the concentration of bicarbonate is low (~2 mM). However, if the concentration of bicarbonate is high (i.e. >20 mM), as is predicted for the Archean ocean (Grotzinger & Kasting, 1993; Ohmoto *et al.*, 2004), even in an atmosphere containing 800 000 p.p.m. H_2, Fe(II) oxidation by these phototrophs could still have proceeded at appreciable rates.

Implications for banded iron formations and metabolic evolution

The implication of our results for BIF genesis is that when the physiological electron acceptor for photosynthesis (CO_2) is abundant (as is presumed to have been the case in an Archaean ocean), the presence of significant quantities of H_2 in the atmosphere would not have precluded Fe(II) oxidation by anoxygenic phototrophs. The fact that the 'high' bicarbonate concentration used in our experiments (20 mM) is lower than its estimated concentration in an Archean ocean by ~3.5-fold implies that the decrease in the Fe(II) oxidation rate reported here may overestimate the inhibition (if any) that would have occurred in the Archean.

The amount of Fe(III) minerals that could have been precipitated in an ancient ocean by anoxygenic phototrophs can be estimated using a rate of Fe(II) oxidation of 0.014 mM Fe(II) day^{-1} (Kappler *et al.*, 2005). Higher rates of phototrophic Fe(II) oxidation have been published recently (Hegler *et al.*, 2008), however, we chose the most conservative rate of Fe(II) oxidation for this calculation. Assuming an area equivalent to that covered by the Hamersley Basin in Western Australia [10^{11} m^2] (Konhauser *et al.*, 2002), $9.0 * 10^{12}$ mol Fe $year^{-1}$ could have been oxidized and then precipitated by anoxygenic phototrophs. For organisms like SW2, the strain whose Fe(II) oxidation activity is most impacted by a H_2 atmosphere (20% relative to its rate in the absence of H_2), $1.8 * 10^{12}$ mol Fe $year^{-1}$ could have been expected to be deposited. For organisms like strain TIE-1, which oxidizes Fe(II) more slowly than SW2 in the absence of H_2 but is less affected by the presence of H_2 (~57% activity relative to its rate in the absence of H_2), an iron deposition rate of $5.1 * 10^{12}$ mol Fe $year^{-1}$ could have been expected. These rates of Fe(II) oxidation are both the same order of magnitude as the maximum rate necessary to deposit the Hamersley BIF ($4.5 * 10^{12}$ mol Fe $year^{-1}$; (Konhauser *et al.*, 2002)). Moreover, these rates assume conditions where H_2 has maximal effect. While these simple calculations are limited because they are based on experiments with only two strains and make an assumption about the baseline Fe(II) oxidation rate relevant for such organisms, to a first approximation, these 'palaeo-ecophysiological' studies support the plausibility of Fe(II)-oxidizing phototrophs in the deposition of Archean BIFs in the presence of an atmosphere with an appreciable amount of H_2.

Given that diffusion and H_2 consumption rates by other bacteria are not considered in this calculation, we expect that the concentration of H_2 would have been even lower within the photic zone inhabited by a community of ancient Fe(II)-oxidizing phototrophs. Additionally, the solubility of H_2 in water decreases with increasing temperature (Fernandez-Prini *et al.*, 2003). If estimations of Archean ocean temperatures at 70 ± 15 °C are correct (Knauth & Lowe, 2003), the concentrations of H_2 seen by SW2 and TIE-1 in our experiments likely grossly over-estimate the amount of H_2 that would have been encountered by their ancient relatives. Therefore, it is likely that at depths approaching 100 m in an ancient ocean, H_2 would have posed no barrier to Fe(II) oxidation. Moreover, in sulfide-depleted environments, which

are thought to have prevailed in the ancient oceans prior to 1.8 Ga (Poulton *et al.*, 2004), Fe(II) may have been the predominant inorganic electron donor available for anoxygenic photosynthesis. Intriguingly, geochemical and stratigraphic data from the 3.4 Ga Buck Reef Chert suggest that the microbial communities active at this time were sustained by H_2 as an electron donor rather than Fe(II) (Tice & Lowe, 2004). This observation, when coupled to our finding that H_2 would have posed no barrier to Fe(II) oxidation, suggests that the metabolic capacity for Fe(II) had not yet evolved at this time (assuming other factors essential for Fe(II) oxidation were not limiting). In this fashion, we can use the logic of palaeoecophysiology to constrain our dating of the rise of particular metabolisms.

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