International Journal of Astrobiology 5 (1): 1–12 (2006) Printed in the United Kingdom
doi:10.1017/S1473550406002886

Nanophase iron oxides as a key ultraviolet sunscreen for ancient photosynthetic microbes

Janice L. Bishop[1,2], Stephanie K. Louris[3], Dana A. Rogoff[1,3] and Lynn J. Rothschild[3,4]

[1]*SETI Institute, 515 N. Whisman Road, Mountain View, CA 94043, USA*
e-mail: jbishop@arc.nasa.gov
[2]*NASA-Ames Research Center, Mail Stop 239-4, Moffett Field, CA 94035, USA*
[3]*NASA-Ames Research Center, Mail Stop 239-20, Moffett Field, CA 94035, USA*
[4]*Stanford University, Program in Human Biology, Stanford, CA 94305, USA*

Abstract: We propose that nanophase iron-oxide-bearing materials provided important niches for ancient photosynthetic microbes on the Earth that ultimately led to the oxygenation of the Earth's atmosphere and the formation of iron-oxide deposits. Atmospheric oxygen and ozone attenuate ultraviolet radiation on the Earth today providing substantial protection for photosynthetic organisms. With ultraviolet radiation fluxes likely to have been even higher on the early Earth than today, accessing solar radiation was particularly risky for early organisms. Yet, we know that photosynthesis arose early and played a critical role in subsequent evolution. Of primary importance was protection below 290 nm, where peak nucleic acid (~260 nm) and protein (~280 nm) absorptions occur. Nanophase ferric oxide/oxyhydroxide minerals absorb, and thus block, the lethal ultraviolet radiation, while transmitting light through much of the visible and near-infrared regions of interest to photosynthesis (400 to 1100 nm). Furthermore, they were available in early environments, and are synthesized by many organisms. Based on experiments using nanophase ferric oxide/oxyhydroxide minerals as a sunscreen for photosynthetic microbes, we suggest that iron, an abundant element widely used in biological mechanisms, may have provided the protection that early organisms needed in order to be able to use photosynthetically active radiation while being protected from ultraviolet-induced damage. The results of this study are broadly applicable to astrobiology because of the abundance of iron in other potentially habitable bodies and the evolutionary pressure to utilize solar radiation when available as an energy source. This model could apply to a potential life form on Mars or other bodies where liquid water and ultraviolet radiation could have been present at significant levels. Based on ferric oxide/oxyhydroxide spectral properties, likely geologic processes, and the results of experiments with the photosynthetic organisms, *Euglena sp.* and *Chlamydomonas reinhardtii*, we propose a scenario where photosynthesis, and ultimately the oxygenation of the atmosphere, depended on the protection of early microbes by nanophase ferric oxides/oxyhydroxides.

Received 28 February 2006, accepted 27 April 2006

Key words: banded iron formations, early Earth, iron oxides, optical spectroscopy, oxygenated atmosphere, photosynthesis, ultraviolet radiation.

Introduction

The atmosphere on most planets is anoxic because of geochemical reactions with surface rocks, whereas an oxic atmosphere generally requires biological activity (Sleep 2001; Catling & Claire 2005; Sleep 2005). One of the unanswered questions about the early Earth is why atmospheric O_2 first rose to higher levels ~2.0–2.45 Ga, as determined by S isotope studies (Farquhar *et al.* 2002; Pavlov 2002; Kasting & Catling 2003), when oxygenic photosynthesis existed much earlier, 2.5 to possibly even 3.8 Ga (Des Marais 2000; Olson 2001; Brocks *et al.* 2003; Rosing & Frei 2004). Recent studies have suggested that methane (Catling *et al.* 2001), sulphur (Kasting *et al.* 1989) or iron (Konhauser *et al.* 2000) could have influenced biogeochemical cycles on the early Earth. Reduced sulphur and/or ferrous iron are thought to be the first electron donors available for early organisms and may have been involved in early photosynthesis (Olson 2001). These anoxigenic photosynthesis reactions probably contributed to the formation of some oxidized ferric iron that may have played a role in early oxygenic photosynthesis and changes in the atmosphere.

Photosynthesis was a critical evolutionary breakthrough; however, photosynthetic organisms faced a dilemma. On the one hand, they needed access to solar radiation for energy; on the other, ultraviolet radiation, especially in the shorter

wavelengths, is extremely damaging. Despite our current atmosphere, which attenuates most radiation below 300 nm, ultraviolet radiation causes DNA damage to life. Even though atmospheric CO_2 would have blocked radiation below 200 nm, without the ozone layer ultraviolet radiation would have posed a substantial burden to early life. The ultraviolet flux to the early Earth was at least as high as it is today, and most likely substantially higher. Although the sun was younger, and about 30 % less luminous, solar ultraviolet radiation from 200 to 320 nm was better able to penetrate this ozone-poor early atmosphere. As the peak absorption for DNA is 260 nm, and that of proteins 280 nm, an increase in solar radiation in the 200 to 320 nm range would have been extremely hazardous to early life, especially forms that would need to access solar energy such as photosynthetic organisms (e.g., Rothschild 1999).

Photosynthesizers were forced to protect themselves either through ultraviolet damage repair or by finding a shield from harmful ultraviolet radiation that still transmits radiation needed for photosynthesis. Radiation that can support photosynthesis is found from ~400 to 1000 nm, although access to the entire range is not necessary. Many kinds of materials could have blocked ultraviolet radiation on the early Earth including iron in general (Olson & Pierson 1986; Pierson *et al.* 1987), ferric oxide/oxyhydroxide (FeOx) minerals (suggested here), sulphur vapour in the atmosphere (Kasting *et al.* 1989), a photochemical haze (Sagan & Chyba 1997; Miller *et al.* 1998), and a combination of inorganic and prebiotic organic compounds in the oceans (Sagan 1973; Cleaves & Miller 1998; Cockell 2000). Towe (1996) suggested that low levels of ozone were present on the early Earth. As a martian biota would face a similar problem with ultraviolet radiation, Sagan and Pollack (1974) suggested that a depth of ~1 cm below the surface on Mars would be a possible endolithic photosynthetic niche, allowing visible light to penetrate while reducing the ultraviolet radiation to acceptable levels. Such communities are known on the Earth, and have been suggested as analogues for martian biota for the reason that they are able to access photosynthetically active radiation (PAR) while being protected from ultraviolet radiation (Rothschild 1995; Rothschild & Giver 2003).

FeOx are unique among the range of possibilities of potential radiation blockers in that they are translucent through much of the visible region (Burns 1993a), they were available in early environments on the Earth (e.g., James & Sims 1973), and are synthesized by many organisms (e.g., Lowenstam 1981). Thus, we suggest here that ferric oxides and oxyhydroxides may be critical in the early evolution of a life-supporting planet as a sunscreen that allowed the evolution of photosynthesis prior to the accumulation of sufficient stratospheric ozone. We have performed several experiments to test our hypothesis that the evolution of some early photosynthetic organisms is related to the presence of nanophase ferric oxide minerals and deposition of iron materials such as red beds or banded iron formations (BIFs).

Background

Iron is geologically abundant, has multiple oxidation states and is arguably the most important metal for life because of its role in many metabolic processes often involving oxygen. Iron is an integral component in the evolution of life. Although iron is the fourth most abundant element on the Earth's crust, it is frequently a limiting factor in biological systems (Falkowski *et al.* 1998) because the bioavailability of iron depends on its redox state and solubility (Martin 1992). The iron balance between organisms and their environment can be viewed as a web, where multiple processes and systems are interlinked and interdependent. The traditionally accepted geologically controlled anoxic-oxic transition (e.g., Des Marais *et al.* 1992) may be sufficient alone to explain many of the processes shaping the evolution of early Earth. However, the possibility exists that biologically mediated processes also played a role in the rise of oxygen. This mix of geological and biological processes could prove to be an important factor in the evolution of habitable worlds.

Aqueous Fe(II) or Fe(III) and iron in mats could have provided ultraviolet protection for photosynthesizers (Olson & Pierson 1986; Pierson *et al.* 1993; Pierson *et al.* 1999; Phoenix *et al.* 2001). Previous studies involving iron and photosynthesis have focused on the morphology of the organisms, chemical interactions of iron with photosynthesis and the abundance of iron (Pierson *et al.* 1999; Pierson & Parenteau 2000; Phoenix *et al.* 2001). In contrast, our study tests whether the spectral properties of specific nanophase-FeOx-bearing minerals could protect photosynthetic organisms from ultraviolet radiation.

Iron oxide and oxyhydroxide minerals

The optical properties of FeOx minerals vary depending on the mineral structure (Burns 1993a). While all FeOx minerals effectively absorb ultraviolet radiation, the spectral properties of nanophase FeOx (that is, very small – of the order of one to hundreds of nanometres in size), in particular, enable good penetration of visible/near-infrared (VNIR) radiation needed by photosynthesizers across a broader wavelength range. Natural Fe-bearing systems are frequently found to contain precipitates of nanophase-FeOx minerals mixed with clays or amorphous silica (Phoenix *et al.* 2001; Bishop & Murad 2002). The type and abundance of nanophase FeOx, clays and/or silica depend on the pH of the water and relative amounts of aqueous Fe, Al and Si (Bigham *et al.* 1996b). One nanophase FeOx, ferrihydrite ($5Fe_2^{3+}O_3 \cdot 9H_2O$), forms ubiquitously in Fe-rich waters and is often a precursor mineral to other FeOx minerals (Cornell & Schwertmann 1996) and was probably present in BIFs. Ferrihydrite is the third most prevalent biogenically produced mineral on Earth (Lowenstam 1981). Nanophase oxyhydroxysulphates (hydrated ferric oxides with sulphate), such as schwertmannite ($Fe_{16}^{3+}O_{16}(OH)_{12}(SO_4)_2 \cdot nH_2O$), form commonly in acid-mine waters and acidic streams (Schwertmann *et al.* 1995; Bigham *et al.* 1996b).

https://doi.org/10.1017/S1473550406002886 Published online by Cambridge University Press

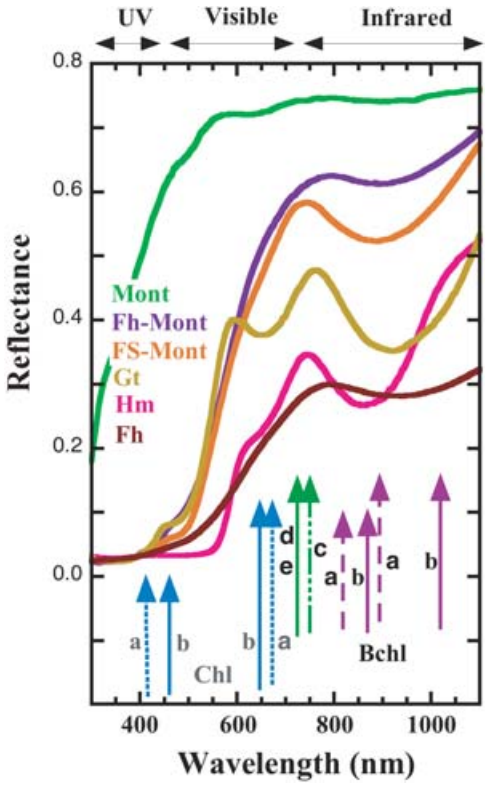


Fig. 1. Reflectance spectra of FeOx and clay powders. Spectra are shown from 300 to 1100 nm of montmorillonite (Mont), ferrihydrite (Fh), ferrihydrite–montmorillonite aggregates (Fh–mont), ferrihydrite–schwertmannite–montmorillonite aggregates (FS–mont), goethite (Gt) and hematite (Hm). The approximate absorption bands are indicated below for chlorophyll (chl) *a* and *b* as well as bacteriochlorophyll (bchl) *a* and *b* in purple bacteria and bacteriochlorophyll *c* and *d*/*e* in green bacteria (Madigan *et al*. 2003).

Nanophase minerals are also frequently formed in the presence of the bacteria *Acidithiobacillus ferrooxidans* (for schwertmannite, pH 2–3.5) and *Gallionella ferruginea* (for ferrihydrite, pH ~7–8), although inorganic formation mechanisms exist as well (e.g., Murad *et al*. 1994; Bigham *et al*. 1996a; Cornell & Schwertmann 1996). Other iron minerals, such as hematite and goethite, could also block ultraviolet radiation; however, these typically form in larger crystalline grains that would be less favourable for coexistence with organisms and they are not as spectroscopically favourable as are the minerals ferrihydrite and schwertmannite.

The spectral properties of iron-oxide-bearing minerals are particularly important to understand in association with the radiation requirements of the microorganisms. Shown in Fig. 1 are extended visible region reflectance spectra of selected iron oxide/oxyhydroxide/oxyhydroxysulphate (FeOx) minerals (Bishop *et al*. 1995; Bishop & Murad 1996). Many FeOx minerals (e.g., hematite and goethite) exhibit strong, narrow absorption features between 600 and 1000 nm due to Fe transitions because of the long-range order in their mineral structures (Bishop *et al*. 1993). In contrast, nanophase-FeOx species (e.g., ferrihydrite and schwertmannite) exhibit broader and less intense absorptions in this region because of a distribution of electronic environments on the Fe. Schwertmannite contains sulphate (Bigham *et al*. 1996b), whereas the others contain only Fe, O, OH and/or H_2O. Fine-grained particles, such as the montmorillonite clay shown in Fig. 1, also absorb radiation well at shorter wavelengths. One potential problem with nanophase FeOx as a sunscreen is that much less radiation is transmitted in the violet to blue, near 400–500 nm, than at longer wavelengths, which is why experiments were needed in order to test our hypothesis that these could have served as a sunscreen that allowed the evolution of photosynthesis prior to the accumulation of sufficient stratospheric ozone. The absorption bands for chlorophyll *a* and *b* (Salisbury & Ross 1992) and the chlorophyll found in green and purple bacteria (Stanier *et al*. 1986) are indicated in Fig. 1 for comparison.

Banded iron formations

BIFs are alternating layers of iron-rich and iron-poor minerals found on the early Earth, and include layers dominated by oxides, siderite (iron carbonate), silicates and sulphides (e.g., James 1954; James & Sims 1973; James & Trendall 1982; Klein 2005). A significant advance in the wealth of organisms on Earth occurred ~2.4 Ga, which was also when a major redox shift towards an oxidizing atmosphere occurred (Kasting 1987; Holland 1999) and when abundant ferric oxide minerals were present. BIFs have been associated on Earth with a major transformation in the redox environment of the oceans and atmosphere, the kinds of organisms present and the surface mineralogy (e.g., Holland 1984; Beukes & Klein 1992; Des Marais 1997; Holland 1999; Catling & Moore 2000; Farquhar *et al*. 2002; Pavlov 2002; Kasting & Catling 2003). If nanophase FeOx were important in the early evolution of photosynthetic organisms, this could explain the approximately synchronous dates of BIF deposition and increases in atmospheric O_2 levels.

The bulk of the BIFs formed rapidly during the mid-Precambrian (~2.0–2.5 Ga) (James & Trendall 1982); however, many formed earlier than 3 Ga (Holland 1984). Although the source and mechanism for these iron formations is not universally accepted, most probably formed in shallow marine environments (Holland 1984), or possibly in evaporite deposits (Eugster & Chou 1973), or due to volcanism (Holland 1984). Ehrenreich and Widdel (1994) have summarized three probable mechanisms of BIF deposition. These include (1) chemical oxidation of dissolved Fe(II) by oxygenic phototrophs (e.g., cyanobacteria) in oxygenated ocean water, (2) abiotic ultraviolet light-driven aqueous oxidation of Fe(II) giving off H_2 and (3) biological oxidation of Fe(II) by anoxygenic photosynthesis. Recent work also re-emphasizes associations between BIF deposition and anoxygenic phototrophic organisms (Kappler *et al*. 2005), Fe(II)-oxidizing photoautotrophic bacteria (Kappler & Newman 2004) and microbial reduction of Fe(III) (Konhauser *et al*. 2005). Localized, ancient iron formations are associated with hydrothermal activity in many locations

https://doi.org/10.1017/S1473550406002886 Published online by Cambridge University Press

documented by Gross (1983), as well as small, current sites in Yellowstone (Pierson *et al.* 1999) and Iceland (Bishop & Murad 2002). Such sites on the early Earth may have provided a convenient habitat for early photosynthesizers, although because chlorophyll breaks down at ~73–75 °C, the settings would have had to be below this temperature range. Holland (1984) supports volcanism as a source of many smaller BIFs, especially those associated with volcanic features, but argues that volcanic processes are unlikely to be responsible for many of the large BIFs because of the enormous volume of iron-rich material in the BIFs, and the relatively short formation time for the bulk of the iron formations. Sleep and Bird (2006) suggest arc volcanoes as one of the most geologically active regions for early microbes.

Iron and photosynthesis

Iron has been linked to the evolution of photosynthesis and the origin of BIFs (Cloud 1973; Pierson & Parenteau 2000). Cloud (1973) asserts that ferrous iron in solution served as a sponge for the biologically generated oxygen that was formed as a waste product of photosynthesis and which would have been toxic to life if allowed to accumulate. According to Cloud's model, the formation of ferric oxides kept O_2 levels sufficiently moderate for photosynthesizers to live and grow. Sulphur isotope studies also suggest that cyanobacteria evolution took place in an iron- and sulphide-rich environment (Saito *et al.* 2003). Recent studies have shown that some forms of iron stimulate photosynthesis (Pierson 1994; Pierson *et al.* 1999; Pierson & Parenteau 2000) and earlier studies showed that iron could be important in protecting the organisms from ultraviolet radiation (Olson & Pierson 1986; Pierson & Olson 1989). However, these studies have focused on the morphology of the organisms, chemical interactions of the Fe with the photosynthesis process and the abundance of iron, rather than the mineralogy of the ferric oxide/oxyhydroxides, or how the spectral properties of these minerals might benefit the organisms.

Previous work (Emerson & Revsbech 1994a,b; Pierson *et al.* 1999; Pierson & Parenteau 2000) has shown that many organisms are intimately clustered with amorphous or nanophase iron oxides on the scale of a few micrometres (Emerson & Revsbech 1994a, b). FeOx minerals that are produced via biologically mediated processes are typically nanophase in scale. This small size would have facilitated the formation of niches for microorganisms because of the high surface area. If nanophase FeOx did provide a protective niche for photosynthetic organisms, as we propose here, this could have allowed for a much more rapid evolution of these organisms than otherwise possible. This would have implications for the anoxic–oxic transition of the planet.

The spectral requirements of photosynthetic organisms depend on the kind of chlorophyll and other pigments used and other chemical factors (e.g., Stanier *et al.* 1986; Rowan 1989; Salisbury & Ross 1992). The first photosynthesizers are thought to have been photoautotrophs performing CO_2-fixation involving reactions with sulphur (Olson & Pierson 1986). They further suggest that the earliest photosynthetic organisms used chlorophyll *a* as the primary e^- donor and that this might have been coupled with Fe–S complexes as e^- acceptors. Another study found five different layers of photosynthetic organisms in a multi-layered microbial salt marsh mat (Pierson *et al.* 1987). In this study the type and abundance of chlorophyll present varied with depth in the mat: chlorophyll *a* (maximum absorption at 665 nm) was found in the upper mat layers (~1–3 mm depth), followed by bacteriochlorophyll *a* (maximum absorption at 773 nm) at ~2–4 mm depth, bacteriochlorophyll *b* (maximum absorption at 790 nm) at ~4–6 mm depth and bacteriochlorophyll *c* (maximum absorption at 669 nm) at ~5–7 mm depth. The observations from this study suggest that layering of materials with different spectral properties can provide multiple niches for distinct populations within one locality. The spectral character of ferric oxide, oxyhydroxide and oxyhydroxysulphate minerals vary depending on their structure and could be formed in layers in mats to the benefit of multiple phototrophic organisms.

Methods

Spectroscopic measurements

Reflectance spectra were measured using a bidirectional spectrometer at Brown University with 5 nm spectral sampling of bulk powders prepared for previous studies (Bishop *et al.* 1993, 1995; Bishop & Murad 2002). Absorbance spectra were measured of the sample suspensions in a 96-well ultraviolet-transparent quartz plate (Corning cat No. 3635) using a Molecular Devices Corp. (Sunnyvale, CA) SpectraMax Plus 384 spectrophotometer. Spectra were measured from 200 to 1000 nm at 2 nm intervals using H_2O as a blank. Typically spectra were measured of four aliquots for each sample and then averaged.

Organisms and culture media

Two protists were used in these experiments as examples of ultraviolet intolerant photosynthetic microbes. *Euglena sp.* was grown in Hutner's medium (Hutner *et al.* 1966) (ATCC Culture Medium 351). *Chlamydomonas reinhardtii* Dangeard was grown in TAP medium (Gorman & Levine 1965).

Protocol for laboratory growth experiments

A suspension of ferrihydrite–montmorillonite aggregates was used for preliminary experiments to test possible toxicity. Sample aliquots of the culture plus growth medium were collected for cell counts before the iron-oxide material was added, 20 min after the addition of iron oxide, again several hours later and finally 4 days later. The samples were illuminated for 24 h in a constant light incubator at 21 °C with ~30 μmol m^{-2} s^{-1} PAR over the range 400–750 nm. Visual inspection after 4 days showed a green colour indicating that abundant organisms survived. The cell counts showed a continual increase in the number of organisms; thus, 10 mg l^{-1} of the ferrihydrite–clay suspensions were not toxic for the *C. reinhardtii* or *Euglena* as hoped.

https://doi.org/10.1017/S1473550406002886 Published online by Cambridge University Press

Another set of preliminary experiments was performed in order to determine what concentration of iron-oxide material to use in our experiments. For these tests four replicates each of ferrihydrite–montmorillonite, ferrihydrite–schwertmannite–montmorillonite, montmorillonite alone and water were prepared with and without *C. reinhardtii* or *Euglena* media in 96-well spectrometer plates and grown in the constant light incubator. These tests showed that concentrations of 2.5, 5.0 and 50 mg l^{-1} suspensions of these iron-bearing materials were safe for the organisms. Based on the absorbance properties of these aggregate suspensions, a concentration of 15 mg l^{-1} was selected for the growth experiments.

Final laboratory growth experiments were performed for a 6-day period. Suspensions were prepared as described above with four mineral samples and water as a control. Culture medium was added to each sample suspension and grown under continual light in an incubator at 21 °C at ~80 μmol PAR. A sample aliquot was removed daily for measurement of the spectral properties and determination of the organism abundance with a hemocytometer.

Protocol for solar radiation experiments

Outdoor experiments were conducted on *Euglena* and *C. reinhardtii*. Fresh medium for each protist was added to five sample suspensions: ferrihydrite–montmorillonite, ferrihydrite–schwertmannite–montmorillonite, montmorillonite alone, ferrihydrite alone and water. These were grown to log phase cultures in the 21 °C constant light incubator described above, at which point aliquots of each sample were removed for absorbance spectra and cell counts. The samples were then enclosed in Whirl-pak® bags (Nasco, Pittsburgh, PA, USA) and floated in a water bath cooled to 25 °C for 4 h at midday under direct sunlight. The total ultraviolet-A (J cm^{-2}, 320–400 nm) and ultraviolet-B (mJ cm^{-2}, 290–320 nm) exposure of the samples was measured using a radiometer (PMA2100; Solar Light Company, Glenside, PA, USA). The Whirl-pak® bags screened ~11% of ultraviolet-A and ~15% of ultraviolet-B radiation in these experiments. Following 4 h of direct sunlight the sample bags were collected, wrapped in foil to prevent further exposure to radiation and placed in the 21 °C constant light incubator overnight. This was performed in order to retain the organisms in a controlled 21 °C environment, while preventing any visible radiation from reaching the samples before the next dose of solar radiation. Aliquots of each sample were again removed for absorbance spectra and cell counts, then the bags were returned to the 25 °C water bath for another 4 h of direct solar radiation. This was repeated for several days until there was evidence for a decrease in green pigment via visual inspection and/or chlorophyll bands in the absorbance spectra.

For one experiment an additional constant light incubator phase was added at the end of the solar radiation experiment in order to determine whether or not the samples had died in the solar radiation experiments. Following several days of the procedure described above for *Euglena* in suspensions of ferrihydrite–montmorillonite, ferrihydrite–schwertmannite–montmorillonite, montmorillonite alone and water, the green colour and chlorophyll spectral features had disappeared. In this case fresh media was added to the sample bags and these were placed in the constant light incubator without foil in order to enable growth of any live cells. The bags were checked daily for evidence of green colour and aliquots were removed for absorbance spectra after 8 days.

Trypan Blue counting technique

Cell viability and abundance were determined by mixing equal volumes of Trypan Blue staining solution (0.4%) (Sigma, Beverly, MA, USA) and the cell culture. Dead cells took up the stain, while live cells did not. After exposing the organisms to the stain for 5 min, the organisms were counted with a Zeiss Axioscope using a hemocytometer for enumeration.

Results

Ultraviolet screening potential of nanophase-FeOx species

Laboratory experiments were performed in order to compare the relative ultraviolet screening abilities of nanophase-FeOx species, selected based on their VNIR optical properties, with those of clay and water. The samples tested included 15 mg l^{-1} suspensions of two nanophase FeOx and clay aggregates, ferrihydrite, montmorillonite clay or H_2O, plus 20% (volume) medium. Absorbance spectra of these aqueous samples are shown normalized to H_2O in Fig. 2. Note that because the media used contain very minor amounts of organics (e.g., vitamin supplements), they themselves do not attenuate the ultraviolet. These spectra suggest that the mixtures of nanophase FeOx with montmorillonite would provide superior ultraviolet absorption to that of the ferrihydrite or clay alone.

Laboratory growth experiments

Growth experiments were conducted in the laboratory in order to ensure that the desired mineral samples would not adversely affect the organisms and to characterize the spectral properties of the growing cultures. Four mineral samples (plus water as a control) were intermixed with the photosynthetic protists *Euglena* and *C. reinhardtii*. In nature these minerals could form clusters around the organisms or layers above the organisms. The strongest chlorophyll absorption peaked at 674 nm in our spectra. The spectral absorbance at 674 nm was normalized by subtracting a continuum component determined from the average of the spectral absorbances at 640 and 720 nm. This was calculated for all spectra in the growth experiments, and selected normalized 674 nm absorbance values are shown in Fig. 3. The chlorophyll bands were typically stronger in the spectra of the *C. reinhardtii* samples than in the spectra of the *Euglena* samples. The absorbance of the chlorophyll bands for all groups of both sets of samples increased with time initially, then became somewhat constant. These data show that the chlorophyll absorbance increased just as readily in spectra of the FeOx

https://doi.org/10.1017/S1473550406002886 Published online by Cambridge University Press

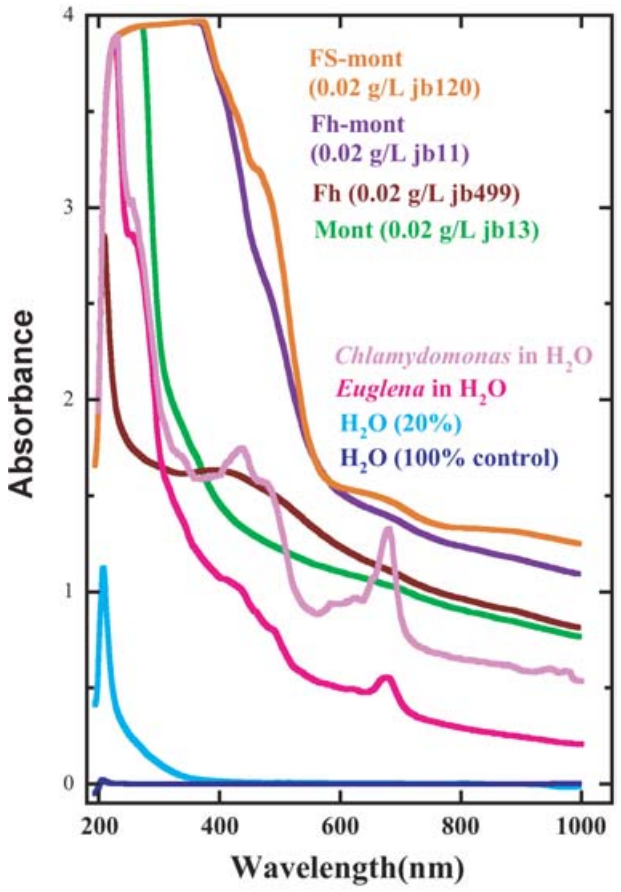


Fig. 2. Absorbance spectra of cultures with mineral admixtures. Spectra are shown of potential sunscreening minerals (definitions as in Fig. 1; jb11, jb13, jb120 and jb499 refer to sample numbers from Bishop *et al.* 1993, 1995; Bishop & Murad 2002) in *C. reinhardtii* medium, and of *Euglena* and *C. reinhardtii* in suspensions with H_2O and media, and of solutions of H_2O and media.

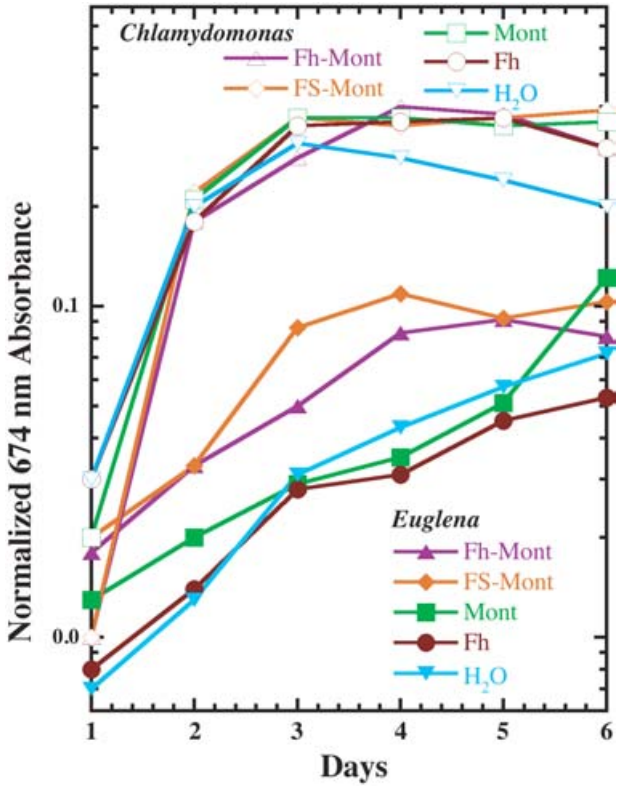


Fig. 3. Normalized absorbance at 674 nm versus growth of organisms in days. The normalized absorbance of chlorophyll *a* at 674 nm is shown for suspensions (definitions as in Fig. 1) of *C. reinhardtii* and *Euglena* grown in a laboratory PAR incubator for several days. The normalized absorbance at 674 nm was determined by subtracting the average absorbance at 640 and 720 nm.

species as in spectra of the montmorillonite clay alone or the water, and that the FeOx species therefore do not prevent growth of *Euglena* or *C. reinhardtii*.

Qualitative abundance measurements of the live and dead *Euglena* and *C. reinhardtii* cells as measured with the hemocytometer showed that the decreasing chlorophyll band strength in the spectra is consistent with the decreasing abundance of live cells. Cell counts for the *C. reinhardtii* and *Euglena* in these experiment are given in Table 1 and images of the live and dead *Euglena* and *C. reinhardtii* cells are shown in Fig. 4. The cell counts are compared with the chlorophyll abundance as a function of growth in days in Fig. 5 for the experiments run in the ferrihydrite–montmorillonite suspensions. These data show that a general trend is observed relating the cell counts and strength of the normalized chlorophyll band. A ratio of approximately 7×10^6 cells per normalized 674 nm chlorophyll absorbance was measured for the *Euglena* and $\sim 3 \times 10^7$ cells per normalized 674 nm chlorophyll absorbance was measured for the *C. reinhardtii*.

Solar radiation experiments

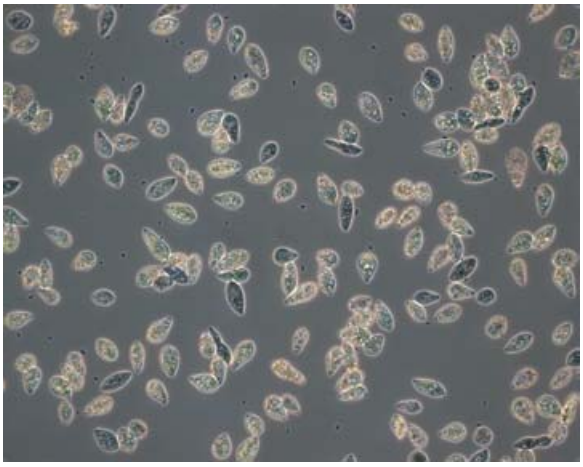
For a more realistic simulation, experiments were conducted to test the sunscreen capacity of nanophase FeOx under natural solar radiation. Both *Euglena* and *C. reinhardtii* are sensitive to ultraviolet exposure and in all of the five sample groups some organisms died in response to the ultraviolet exposure, resulting in loss of green colour by visual inspection, cell destruction observed under phase contrast microscopy and loss of the chlorophyll bands in the absorbance spectra. It is expected that early photosynthetic microbes would also be sensitive to ultraviolet radiation; however, these organisms are highly ultraviolet intolerant, which facilitated experimental study in a timely manner. The cultures containing only H_2O had the highest death rate in both experiments. The samples containing the nanophase FeOx or clay were more resistant to the ultraviolet exposure, and those containing both nanophase FeOx and clay were most resistant to the ultraviolet exposure. Shown in Fig. 6 is a 96-well absorbance plate containing aliquots of the *C. reinhardtii* and *Euglena* samples prior to the solar radiation experiment, following 4 h of ultraviolet radiation on the first day, and again after an additional 4 h of ultraviolet radiation on the next day. The water control in column a and the montmorillonite

https://doi.org/10.1017/S1473550406002886 Published online by Cambridge University Press

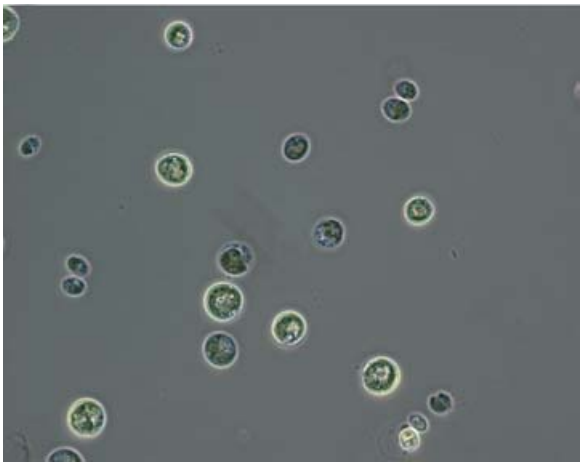
Table 1. *Abundance of live cells measured in the laboratory growth experiment*

Growth (days)	Water	Montmorillonite	Ferrihydrite	Fh–mont	FS–mont
C. reinhardtii					
0	1.70E+07	1.70E+07	1.43E+07	2.60E+07	2.00E+07
2	2.30E+07	2.90E+07	2.25E+07	3.11E+07	1.19E+07
3	2.69E+07	2.42E+07	3.03E+07	2.86E+07	1.24E+07
4	3.28E+07	2.32E+07	2.32E+07	1.51E+07	2.00E+07
5	3.92E+07	3.11E+07	2.74E+07	2.92E+07	2.00E+07
6	4.71E+07	3.73E+07	4.03E+07	3.32E+07	1.82E+07
Euglena					
0	1.03E+06	1.19E+06	4.18E+05	2.28E+06	8.53E+05
2	3.75E+06	7.00E+06	4.25E+06	8.00E+06	3.25E+06
3	5.40E+06	1.67E+07	6.20E+06	9.33E+06	6.00E+06
4	5.14E+06	1.97E+07	7.60E+06	1.28E+07	7.50E+06
5	5.14E+06	1.92E+07	5.33E+06	8.33E+06	8.67E+06
6	3.41E+06	7.33E+06	1.04E+07	1.48E+07	1.14E+07

Fh–mont is Ferrihydrite–montmorillonite assemblage and FS–mont is Ferrihydrite–schwertmannite–montmorillonite assemblage.



(a)



(b)

Fig. 4. Images of live (clear) and dead (coloured) cells following staining with Trypan Blue: (a) *Euglena* magnified 21×; and (b) *C. reinhardtii* magnified 67×.

sample in column c appear green or white, whereas the FeOx samples in columns b, d and e are reddish-orange in colour.

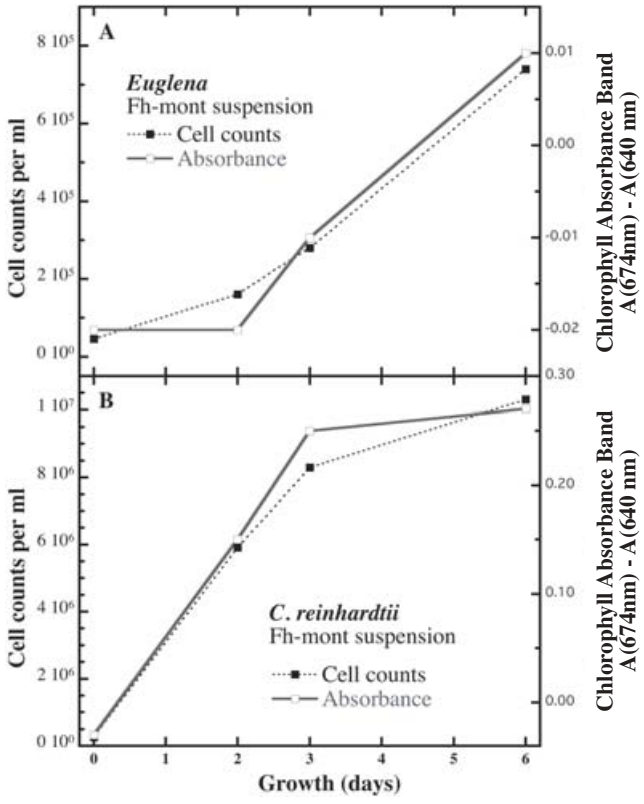


Fig. 5. Comparison of cell counts and chlorophyll absorbance band strength for two samples: (a) *Euglena* and (b) *C. reinhardtii* in ferrihydrite–montmorillonite aggregate (Fh–mont) suspension.

Transmittance spectra of the *C. reinhardtii* suspensions are shown in Fig. 7 measured for the samples before radiation exposure and after each additional exposure to solar radiation. Similar spectra were recorded for the *Euglena* suspensions, but are not shown here. The overall transmittance increased in general with ultraviolet exposure and changes were observed in the chlorophyll bands near 400–500 and 600–700 nm. The chlorophyll band centred near 674 nm is the dominant feature in all of these spectra. The first three spectra for each *C. reinhardtii* suspension in Fig. 7 were taken of

https://doi.org/10.1017/S1473550406002886 Published online by Cambridge University Press

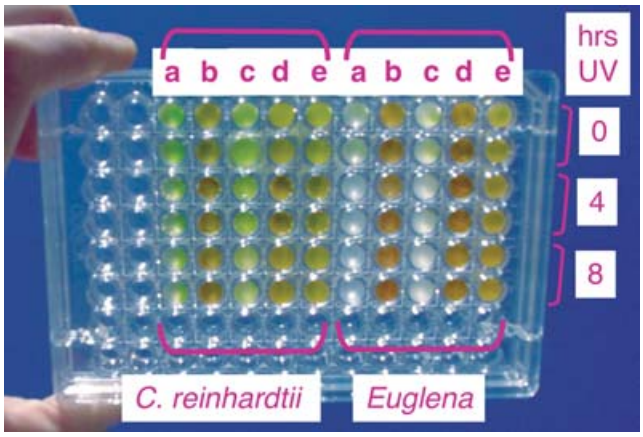


Fig. 6. Image of a 96-well spectrophotometer plate containing *C. reinhardtii* and *Euglena* samples from the solar radiation experiment. The top two rows are replicates of the samples before ultraviolet exposure, the next two rows are replicates of the samples after 4 h of ultraviolet exposure, and the bottom two rows are replicates of the samples after 8 h of ultraviolet exposure. Samples are ordered by column for each organism: (a) H_2O control; (b) ferrihydrite; (c) montmorillonite; (d) ferrihydrite–montmorillonite assemblage; and (e) ferrihydrite–schwertmannite–montmorillonite assemblage.

the samples shown in Fig. 6. A lightening of the green colour in Fig. 6 is correlated with a reduction in chlorophyll absorptions in the spectra shown in Fig. 7. The transmittance of the FeOx samples is close to zero below 500 nm because these samples absorb so strongly at shorter wavelengths and thus have a reddish-orange colour, as seen in Fig. 6. This is not the case for the water control and montmorillonite sample where the weaker chlorphyll bands near 400–500 nm can be observed as well. All of these spectra show decreases in the chlorophyll bands over time with increasing ultraviolet exposure. In order to semi-quantitatively monitor changes in chlorophyll abundance over time in these experiments, normalized 674 nm absorbance for the *C. reinhardtii* suspensions at selected ultraviolet exposure levels are shown in Fig. 8. These data show quantitative decreases in the normalized chlorophyll bands with increasing radiation exposure. Small decreases were observed initially over 4 days of 4 h doses of midday solar radiation, followed by a larger decrease in chlorophyll after the 4 h solar radiation exposure on the fifth day of treatment. The relative changes in chlorophyll abundance for this fifth dose of ultraviolet radiation were most drastic for the samples grown in water or clay only, as shown for the *C. reinhardtii* suspensions in Fig. 8. Although the chlorophyll levels in the *C. reinhardtii* and *Euglena* cultures decreased in these experiments, obvious chlorophyll bands were still present in the nanophase-ferric-oxide-bearing suspensions showing that the organisms survived with the protection of these minerals. The ultraviolet-A and ultraviolet-B exposure levels of the samples are shown in Fig. 9. The ultraviolet-A and ultraviolet-B levels measured were fairly constant for the experiment days and were typical of cloudless summer days in Mountain View, CA. That the chlorophyll levels dropped after the fifth dose of ultraviolet radiation does not appear to be due to any sudden increase in ultraviolet that day, but rather to the cumulative effect of solar radiation in the experiment.

In an additional experiment, *Euglena* cultures were exposed to solar radiation at midday for 4 days as described above until all samples lost their green colour and the chlorophyll bands were absent or extremely weak. Transmittance spectra of the *Euglena* suspensions are shown in Fig. 10. Increasing transmittance and weakening of the chlorophyll bands were observed in spectra of the *Euglena* suspensions with increasing solar radiation exposure in these experiments. During this experiment the radiation levels were approximately 3–3.5 mW cm^{-2} ultraviolet-A and 10^{-13} μW cm^{-2} ultraviolet-B at midday. It was assumed that by the end of 4 days of ultraviolet exposure in this experiment the microbes were severely stressed and perhaps dying or dead. The samples were then returned to the laboratory incubator for 8 days in order to determine if the *Euglena* cultures would recover again in an ultraviolet-free environment. The nanophase-FeOx–clay sample cultures turned green and exhibited a substantial increase in the spectral bands due to chlorophyll during this post-experiment laboratory growth trial, while the water and montmorillonite clay samples did not. The chlorophyll bands in the post-solar-incubator-treated sample spectra can be seen for the ferrihydrite–montmorillonite and ferrihydrite–schwertmannite–montmorillonite suspensions shown in Fig. 10(c) and (d). Normalized chlorophyll absorbance levels for these experiments are shown in Fig. 11. Continual decreases in the chlorophyll abundance are observed in the spectra of all samples; however, the loss of chlorophyll is strongest for the water only sample, next for the clay only sample and least for the ferrihydrite–montmorillonite and ferrihydrite–schwertmannite–montmorillonite samples. As seen in Fig. 11, following the rehabilitation effort in the laboratory incubator, the water only sample showed no chlorophyll present, the montmorillonite sample showed no increase (constant very low chlorophyll level) and the ferrihydrite–montmorillonite and ferrihydrite–schwertmannite–montmorillonite samples showed marked increases in chlorophyll levels. These experiments show that the nanophase FeOx imbedded in montmorillonite clay provided an effective sunscreen for *Euglena* that enabled them to survive solar radiation much longer than clay or water alone.

Discussion

We selected four mineral assemblages as potential sunscreening agents for experiments with *Euglena* and *C. reinhardtii* based on the visible and near-infrared spectral properties of these minerals. The nanophase-FeOx mineral ferrihydrite and the clay mineral montmorillonite were used as well as a ferrihydrite–montmorillonite assemblage and a ferrihydrite–schwertmannite–montmorillonite assemblage. Schwertmannite is a nanophase mineral containing sulphate as well as hydrated ferric oxide. Tests were run in the laboratory in a constant light PAR incubator to ensure that

https://doi.org/10.1017/S1473550406002886 Published online by Cambridge University Press

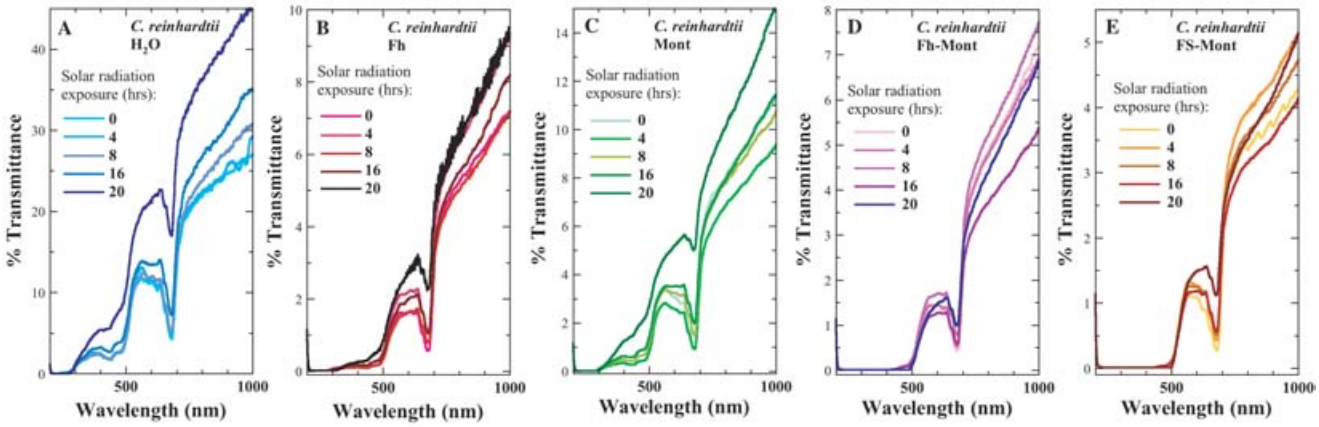


Fig. 7. Transmittance spectra of *C. reinhardtii* suspensions exposed to natural solar radiation over several days: (a) H_2O control; (b) ferrihydrite; (c) montmorillonite; (d) ferrihydrite–montmorillonite assemblage; and (e) ferrihydrite–schwertmannite–montmorillonite assemblage. Decreases in the chlorophyll bands are observed with increasing solar radiation as the *C. reinhardtii* are stressed from the ultraviolet radiation.

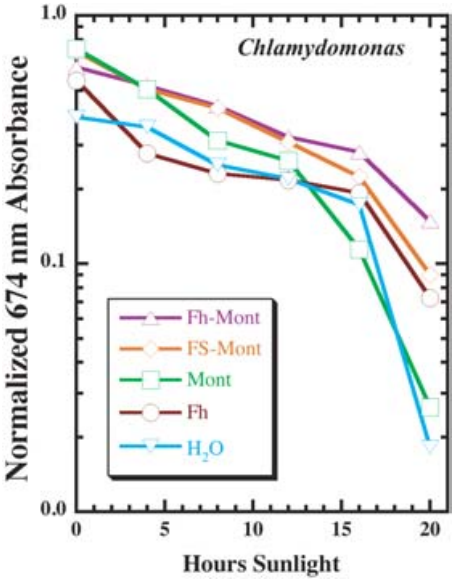


Fig. 8. Normalized absorbance of chlorophyll *a* at 674 nm in spectra of *C. reinhardtii* suspensions (definitions as in Fig. 1) exposed to natural solar radiation in small doses over several days.

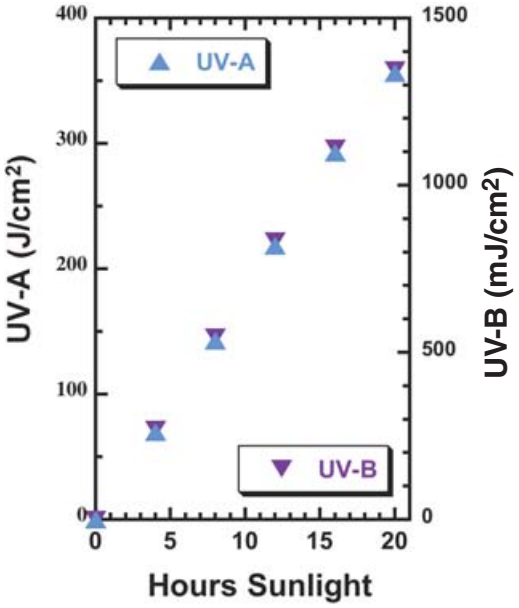


Fig. 9. The cumulative ultraviolet-A and ultraviolet-B levels for the *Chlamydomonas* experiment.

the *Euglena* and *C. reinhardtii* could thrive in suspensions of these minerals and mineral assemblages. Solar radiation experiments with *Euglena* and *C. reinhardtii* in suspensions of these minerals and mineral assemblages showed that all four minerals/mineral assemblages helped protect the organisms compared with the water control. However, the organisms grown in suspensions of the ferrihydrite–montmorillonite assemblage and the ferrihydrite–schwertmannite–montmorillonite assemblage survived longer under stressed solar ultraviolet radiation exposure conditions. The ~674 nm chlorophyll spectral band strength of the samples was found to be well correlated with the abundance of live organisms measured via cell counts. In a subsequent experiment with the *Euglena*, where the samples were placed in the constant light PAR incubator following the ultraviolet experiment that killed off many organisms, the samples grown in the ferrihydrite–montmorillonite assemblage and the ferrihydrite–schwertmannite–montmorillonite assemblage were able to grow again and produce chlorophyll, while the samples grown in montmorillonite clay and water were unable to do so.

Possible photosynthetic niches on Mars

Iron oxides are also pervasive on Mars (Bell *et al.* 1990). In particular, the dust covering the planet contains substantial amounts of nanophase FeOx (Morris *et al.* 1989; Bishop *et al.* 1993). Recent evidence for persistent aqueous activity at Meridiani Planum on Mars (Squyres *et al.* 2004) opens

https://doi.org/10.1017/S1473550406002886 Published online by Cambridge University Press

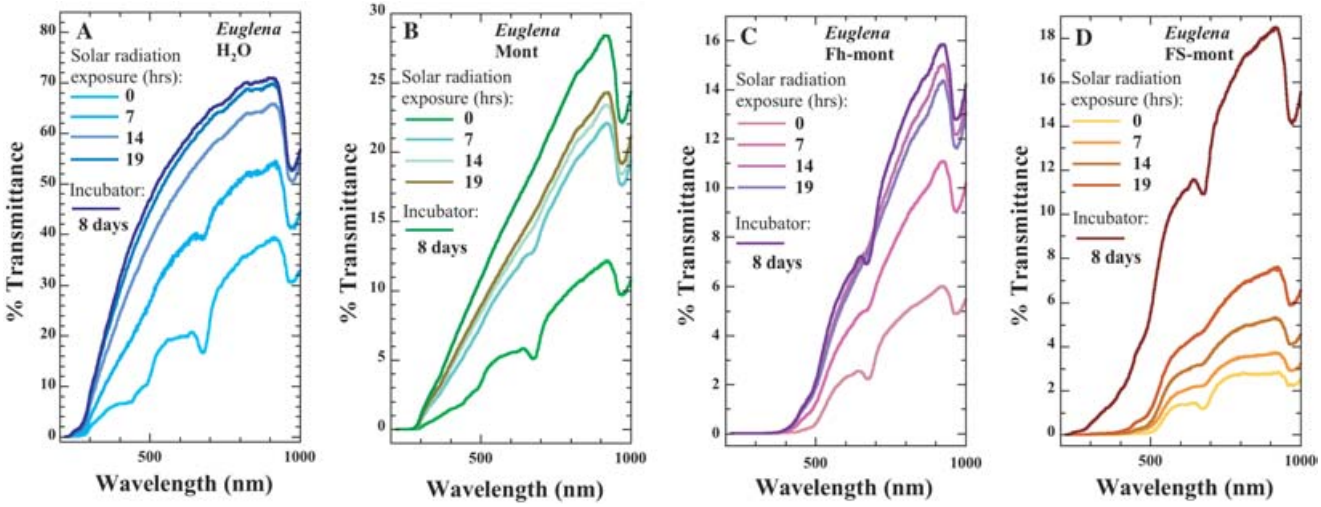


Fig. 10. Transmittance spectra of *Euglena* suspensions exposed to natural solar radiation over several days: (a) H_2O control; (b) montmorillonite; (c) ferrihydrite–montmorillonite assemblage; and (d) ferrihydrite–schwertmannite–montmorillonite assemblage. Decreases in the chlorophyll bands are observed with increasing solar radiation as the *Euglena* are stressed from the ultraviolet radiation. An additional spectrum was measured after growing the stressed organisms in the laboratory incubator for 8 days. This final spectrum showed a return of the chlorophyll band at 674 nm for the FeOx–clay samples shown in (c) and (d).

the door to the possibility of life there. Given abundant water and nanophase FeOx, habitats could have existed for photosynthesis on Mars.

Burns (1993b) first suggested the possibility of abiotic BIF-like materials on Mars in the form of desiccated FeOx/silica deposits. Schaefer (1996) discussed the potential formation of BIFs on Mars through several abiotic mechanisms including oxidation of ferrous iron by free oxygen, photooxidation of ferrous iron, oxidation of ferrous iron by H_2O_2 and anoxic precipitation of ferrous iron by changes in pH. In an aqueous environment such BIFs could have presented habitable niches for photosynthetic microbes on Mars. H_2O_2 could have also been important in drier environments. More recently, Calvin (1998) suggested that BIFs on Mars may have precipitated ferrous clays and other dark ferrous minerals and provided a model to explain the dark regions on Mars through a BIF-like mechanism. As these ferrous minerals are exposed to the oxidizing surface they would convert to ferric minerals including hematite.

In a pre-Viking assessment of the possibility of life on Mars, Sagan and Lederberg (1976) argued that liquid water is the most stringent limiting factor for life on Mars. They suggested that the low oxygen partial pressure would be a negligible impediment to life and that the ultraviolet flux could have been countered by living below the surface or by developing an ultraviolet-absorbing exoskeleton or shield (that might also be able to conserve water). Such speculation has been largely forgotten since the Viking biology experiments did not result in any evidence for the presence of life (e.g., Klein 1978). Weiss *et al.* (2000) recently emphasized the availability of liquid water on Mars as potentially the most important limiting factor for living organisms. Their study showed that the apparent scarcity of life on Mars could not be attributed to a lack of energy. With the recent geomorphologic evidence

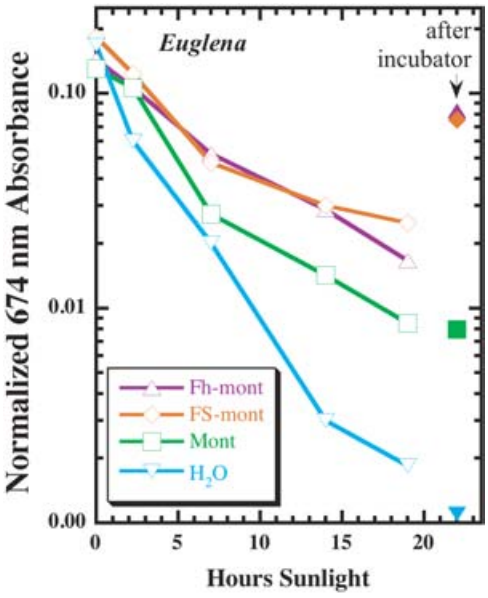


Fig. 11. Normalized absorbance of chlorophyll *a* at 674 nm in spectra of *Euglena* suspensions (definitions as in Fig. 1) exposed to natural solar radiation in small doses over several days. An additional spectrum was measured after growing the stressed organisms in the laboratory incubator for 8 days.

supporting frozen aquifers and some liquid water on Mars (Malin & Edgett 2000; Christensen 2003; Squyres *et al.* 2004), it may be timely to re-evaluate the possibility of life on Mars at some point in the planet's history, and the possible niches that may be (or have been) present for life on Mars-like planets. Nealson (1997) has summarized a variety of chemical energy sources for bacteria and described several unusual ecosystems on Earth that may have been possible on Mars (or

https://doi.org/10.1017/S1473550406002886 Published online by Cambridge University Press

other planets) at one time in the past. These include Antarctic endoliths, lakes in the Antarctic Dry Valleys and deep subsurface ecosystems. If our hypothesis that nanophase FeOx could provide a protective habitat for photosynthetic organisms is correct, then Mars-like planets with abundant FeOx and some liquid water may have been (be) able to support life.

Possible links between nanophase FeOx and oxygenic photosynthesis

Prior to the evolution of oxygenic photosynthesis on Earth, chemoautotrophs and anoxygenic photosynthesis were present (e.g., Holland 1984). Anoxic photosynthesis is thought to have emerged by ~2.8 Ga (Xiong *et al.* 2000). It may have taken place at depth using water to insulate the organisms from ultraviolet radiation. These may have lived near volcanic vents, which could have provided warmth and nutrients for the photochemical reactions (Nisbet & Sleep 2001). Aqueous nanophase-FeOx deposits generated by photolysis and oxidation of Fe(II) (perhaps including smaller hydrothermal BIFs) may have provided ideal niches for anoxic photosynthesis, which in turn produced small pockets of FeOx for oxygenic photosynthesis and eventually larger BIFs. Konhauser *et al.* (2005) suggest strong links between microbial Fe(III) reduction and BIF deposition. Oxidation of Fe(II) by multiple oxides of nitrogen (probably also resulting from photosynthesis) could have also served as a source of Fe(III) for formation of ferric oxides (Summers & Chang 1993; Summers 1999). Perhaps the FeOx minerals that formed the BIFs enabled the then new photosynthesis mechanism to become more widespread. Current biogeochemical modelling approaches may also provide insights into how organisms have shaped and are shaped by their environments (Newman & Banfield 2002). It is likely that oxygenic photosynthesis occurred at least 600 Myr prior to the time of high atmospheric O_2 levels (Des Marais *et al.* 1992; Saito *et al.* 2003). The negative influence of solar radiation may have initially hindered the evolution of photosynthesis, until appropriate FeOx sunscreening niches were found. If our hypothesis is correct, then the presence of nanophase FeOx could have significantly enhanced survival rates of photosynthetic organisms, thereby linking FeOx to an increase in atmospheric O_2 levels.

As outlined by Catling and Claire (2005), production of O_2 in isolated regions on the early Earth would have produced little change in the planet's atmosphere, as long as local geochemical processes were able to absorb this O_2. In fact, if the early atmosphere contained methane, this methane would have hindered the buildup of O_2 in the atmosphere. Possibly, then, photosynthesis could have taken place where these organisms were protected by nanophase FeOx. These higher oxygen levels would have formed larger FeOx deposits, making more and larger niches for photosynthesis. When the O_2 production exceeded the geologic reducing power, then the atmosphere became oxic (Holland 1984). This could have led to the development of expansive BIFs, widespread oxygenic photosynthesis and an oxygenated atmosphere. This is thought to have occurred by ~2.3 Ga (Des Marais 2000; Kasting 2001). Our experiments support nanophase FeOx as novel and unique solar ultraviolet radiation shields that may have played a role in the early evolution of photosynthesis on Earth and may be linked to the formation of BIFs. As nanophase FeOx are ubiquitous on Mars, this scenario may have relevance there as well.

Summary and conclusions

Atmospheric oxygen and ozone attenuate ultraviolet radiation on Earth today providing substantial protection for photosynthetic organisms. However, early photosynthesizers lacked this atmospheric protection. Nanophase-FeOx minerals absorb, and thus block, the lethal ultraviolet radiation, while transmitting light through much of the visible and infrared regions. Our experiments showed that nanophase-FeOx materials in suspensions with photosynthetic microbes provided them with sufficient ultraviolet protection to live longer in a hostile ultraviolet environment than non-protected organisms. Mixtures of the nanophase-FeOx minerals ferrihydrite or schwertmannite with the clay mineral montmorillonite provided better protection in our experiments than either the montmorillonite or ferrihydrite alone. Based on the spectral properties of FeOx and the results of our experiments with the photosynthetic organisms *Euglena* and *C. reinhardtii*, we propose a scenario where photosynthesis, and ultimately the oxygenation of the atmosphere, depended on the protection of early microbes by nanophase FeOx. Early iron deposits on Earth such as red beds and BIFs may also be related to the evolution of oxygenic photosynthesis and thus the oxygenation of the atmosphere. Similar iron-dependent ultraviolet-protected niches may have also existed on Mars and other surfaces in the solar system and provided a habitat for oxygenic photosynthesis.

Acknowledgements

The authors are grateful to the NASA Astrobiology Institute for support of this project. Helpful comments from N. Sleep and M. Schaefer are much appreciated.

References

Bell, J.F. III, McCord, T.B. & Owensby, P.D. (1990). *J. Geophys. Res.* **95**, 14 447–14 461.

Beukes, N.J. & Klein, C. (1992). *The Proterozoic Biosphere*, eds Schopf, J.W. & Klein, C., pp. 147–152. Cambridge University Press, New York.

Bigham, J.M., Schwertmann, U. & Pfab, G. (1996a). *Appl. Geochem.* **11**, 845–849.

Bigham, J.M., Schwertmann, U., Traina, S.J., Winland, R.L. & Wolf, M. (1996b). *Geochim. Cosmochim. Acta* **60**, 2111–2121.

Bishop, J.L. & Murad, E. (1996). *Mineral Spectroscopy: A Tribute to Roger G. Burns*, eds Dyar, M.D., McCammon, C. & Schaefer, M.W., pp. 337–358. The Geochemical Society, Houston, TX.

Bishop, J.L. & Murad, E. (2002). *Volcano–Ice Interactions on Earth and Mars*, eds Smellie, J.L. & Chapman, M.G., pp. 357–370. Geological Society, Special Publication No. 202, London.

Bishop, J.L., Pieters, C.M. & Burns, R.G. (1993). *Geochim. Cosmochim. Acta* **57**, 4583–4595.
Bishop, J.L., Pieters, C.M., Burns, R.G., Edwards, J.O., Mancinelli, R.L. & Froeschl, H. (1995). *Icarus* **117**, 101–119.
Brocks, J.J., Buick, R., Summons, R.E. & Logan, G.A. (2003). *Geochim. Cosmochim. Acta* **67**, 4321–4335.
Burns, R.G. (1993a). *Mineralogical Applications of Crystal Field Theory*. Cambridge University Press, Cambridge.
Burns, R.G. (1993b). *Geochim. Cosmochim. Acta* **57**, 4555–4574.
Calvin, W.M. (1998). *Geophys. Res. Lett.* **25**, 1597–1600.
Catling, D. & Claire, M.W. (2005). *Earth Planet. Sci. Lett.* **237**, 1–20.
Catling, D. & Moore, J.G. (2000). *Lunar Planet. Sci.* XXXI. CD-ROM #1517 (abstr.). Lunar Planetary Institute, Houston.
Catling, D.C., Zahnle, K.J. & McKay, C.P. (2001). *Science* **293**, 839–843.
Christensen, P.R. (2003). *Nature* **422**, 45–48.
Cleaves, H.J. & Miller, S.L. (1998). *Proc. Nat. Acad. Sci., USA* **95**, 7260–7263.
Cloud, P. (1973). *Economic Geology* **68**, 1135–1143.
Cockell, C.S. (2000). *Origins of Life and Evolution of the Biosphere* **30**, 467–499.
Cornell, R.M. & Schwertmann, U. (1996). *The Iron Oxides*. VCH, New York.
Des Marais, D.J. (1997). *Organic Geochem.* **27**, 185–193.
Des Marais, D.J. (2000). *Science* **289**, 1703–1705.
Des Marais, D.J., Strauss, H., Summons, R.E. & Hayes, J.M. (1992). *Nature* **359**, 605–609.
Ehrenreich, A. & Widdel, F. (1994). *Appl. Environ. Microbiol.* **60**, 4517–4526.
Emerson, D. & Revsbech, N.P. (1994a). *Appl. Environ. Microbiol.* **60**, 4022–4031.
Emerson, D. & Revsbech, N.P. (1994b). *Appl. Environ. Microbiol.* **60**, 4032–4038.
Eugster, H.P. & Chou, I.-M. (1973). *Economic Geology* **68**, 1144–1168.
Falkowski, P.G., Barber, R.T. & Smetacek, V. (1998). *Science* **281**, 200–206.
Farquhar, J., Wing, B.A., McKeegan, K.D., Harris, J.W., Cartigny, P. & Thiemens, M.H. (2002). *Science* **2998**, 2369–2372.
Gorman, D.S. & Levine, R.P. (1965). *Proceeding of the National Academy of Sciences* **54**, 1665.
Gross, G.A. (1983). *Precambrian Research* **20**, 171–187.
Holland, H.D. (1984). *The Chemical Evolution of the Atmosphere and Oceans*, Princeton University Press, Princeton.
Holland, H.D. (1999). *Geochem. News* **100**, 20–22.
Hutner, S.H., Zahalsky, A.C., Aaronsen, S., Baker, H. & Frank, O. (1966). *Methods Cell Physiol.* **2**, 217–228.
James, H.L. (1954). *Economic Geology* **49**, 235–293.
James, H L. & Sims, P.K. (1973). *Economic Geology* **68**, 913–914.
James, H.L. & Trendall, A.F. (1982). *Mineral Deposits and the Evolution of the Biosphere*, eds Holland, H.D. & Schidlowski, M., pp. 199–217. Springer-Verlag, New York.
Kappler, A. & Newman, D.K. (2004). *Geochim. Cosmochim. Acta* **68**, 1217–1226.
Kappler, A., Pasquero, C., Konhauser, K.O. & Newman, D.K. (2005). *Geology* **33**, 865–868.
Kasting, J.F. (1987). *Precambrian Research* **34**, 205–229.
Kasting, J.F. (2001). *Science* **293**, 819–820.
Kasting, J.F. & Catling, D. (2003). *Ann. Rev. Astron. Astrophys.* **41**, 429–463.
Kasting, J.F., Zahnle, K.J., Pinto, J.P. & Young, A.T. (1989). *Origins of Life and Evolution of the Biosphere* **19**, 95–108.
Klein, C. (2005). *Amer. Mineralogist* **90**, 1473–1499.
Klein, H.P. (1978). *Icarus* **34**, 666–674.
Konhauser, K., Phoenix, V. & Adams, D. (2000). *Goldschmidt Conference*, Vol. 5(2), p. 597. Cambridge Publications, Oxford, UK.
Konhauser, K.O., Newman, D.K. & Kappler, A. (2005). *Geobiology* **3**, 167–177.
Lowenstam, H.A. (1981). *Science* **211**, 1126–1131.
Madigan, M.T., Martinko, J.M. & Parker, J. (2003). *Brock Biology of Microorganisms*, 10th edn. Prentice-Hall, Pearson Education, Inc., Upper Saddle River, NJ.
Malin, M.C. & Edgett, K.S. (2000). *Science* **288**, 2330–2335.
Martin, J.H. (1992). *Primary Productivity and Biogeochemical Cycles in the Sea*, eds Falkowski, P.G. & Woodhead, A., pp. 123–137. Plenum, New York.
Miller, S.L., Lyons, J.R. & Chyba, C.F. (1998). *Science* **279**, 779a.
Morris, R.V., Agresti, D.G., Lauer, H.V. Jr, Newcomb, J.A., Shelfer, T.D. & Murali, A.V. (1989). *J. Geophys. Res.* **94**, 2760–2778.
Murad, E., Schwertmann, U., Bigham, J.M. & Carlson, L. (1994). *Environmental Geochemistry of Sulphide Oxidation*, eds Alpers, C.N. & Blowes, D.W., pp. 190–200. American Chemistry Society, Washington, DC.
Nealson, K. (1997). *J. Geophys. Res.* **102**, 23 675–23 686.
Newman, D.K. & Banfield, J.F. (2002). *Science* **296**, 1071–1077.
Nisbet, E.G. & Sleep, N.H. (2001). *Nature* **409**, 1083–1091.
Olson, J.M. (2001). *Photosynthesis Research* **68**, 95–112.
Olson, J.M. & Pierson, B.K. (1986). *Photosynthesis Research* **9**, 251–259.
Pavlov, A.A. (2002). *Astrobiology* **2**, 27–41.
Phoenix, V., Konhauser, K., Adams, D.G. & Bottrell, S.H. (2001). *Geology* **29**, 823–826.
Pierson, B.K. (1994). *Early Life on Earth*, ed. Bengtson, S., pp. 161–180. Columbia University Press, New York.
Pierson, B.K., Mitchell, H.K. & Ruff-Roberts, A.L. (1993). *Origins Life Evolution Biosphere* **23**, 243–260.
Pierson, B.K., Oesterle, A. & Murphy, G.L. (1987). *FEMS Microbiol. Ecol.* **45**, 365–376.
Pierson, B.K. & Olson, J.M. (1989). *Microbial Mats. Physiological Ecology of Benthic Microbial Communities*, eds Cohen, Y. & Rosenberg, E., pp. 402–426. American Society of Microbiology, Washington, DC.
Pierson, B.K. & Parenteau, M.N. (2000). *FEMS Microbiol. Ecol.* **32**, 181–196.
Pierson, B.K., Parenteau, M.N. & Griffin, B.M. (1999). *Appl. Environ. Microbiol.* **65**, 5474–5483.
Rosing, M.T. & Frei, R. (2004). *Earth Planet. Sci. Lett.* **217**, 237–244.
Rothschild, L.J. (1995). *Adv. Space Res.* **15**, 223–228.
Rothschild, L.J. (1999). *J. Eukaryot Microbiol.* **46**, 548–555.
Rothschild, L.J. & Giver, L.J. (2003). *Int. J. Astrobiol.* **1**, 295–304.
Rowan, K.S. (1989). *Photosynthetic Pigments of Algae*. Cambridge University Press, New York.
Sagan, C. (1973). *J. Theor. Biol.* **39**, 195–200.
Sagan, C. & Chyba, C. (1997). *Science* **276**, 1217–1221.
Sagan, C. & Lederberg, J. (1976). *Icarus* **28**, 291–300.
Sagan, C. & Pollack, J.B. (1974). *Icarus* **21**, 490–495.
Saito, M.A., Sigman, D.M. & Morel, F.M.M. (2003). *Inorganic Chim. Acta* **356**, 308–318.
Salisbury, F.B. & Ross, C.W. (1992). *Plant Physiology*, Wadsworth Pub. Co., Belmont, CA.
Schaefer, M.W. (1996). *Mineral Spectroscopy: A Tribute to Roger G. Burns*, eds Dyar, M.D., McCammon, C. & Schaefer, M.W., pp. 381–393. The Geochemical Society, Houston, TX.
Schwertmann, U., Bigham, J.M. & Murad, E. (1995). *Euro. J. Mineral.* **7**, 547–552.
Sleep, N.H. (2001). *Nature* **410**, 317–319.
Sleep, N.H. (2005). *Biogeochemical Cycles of Elements* (*Metal Ions in Biological Systems*, Vol. 43), ed. Sigel, A., pp. 49–73. Dekker, Salisbury.
Sleep, N.H. & Bird, D.K. (2006). *Astrobiology* **6**, 110.
Squyres, S.W. *et al.* (2004). *Science* **306**, 1698–1703.
Stanier, R.Y., Ingraham, J.L., Wheelis, M.L. & Painter, P.R. (1986). *The Microbial World*. Prentice-Hall, Englewood Cliffs, NJ.
Summers, D.P. (1999). *Origins Life Evolution Biosphere* **29**, 33–46.
Summers, D.P. & Chang, S. (1993). *Nature* **365**, 630–633.
Towe, K.M. (1996). *Adv. Space Res.* **18**, 7–15.
Weiss, B.P., Yung, Y.L. & Nealson, K.H. (2000). *Proc. Nat. Acad. Sci., USA* **97**, 1395–1399.
Xiong, J., Fischer, W.M., Inoue, K., Nakahara, M. & Bauer, C.E. (2000). *Science* **289**, 1724–1730.