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Bacteriogenic Manganese Oxides

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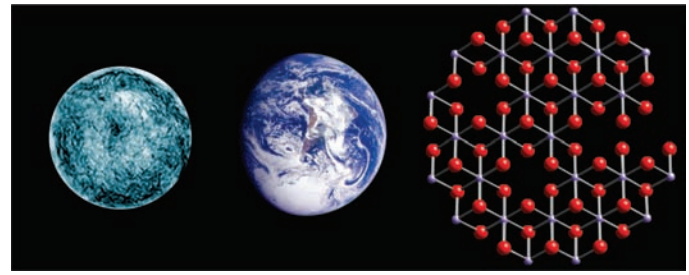

Microorganisms control the redox cycling of manganese in the natural environment. Although the homogeneous oxidation of Mn(II) to form manganese oxide minerals is slow, solid MnO_2 is the stable form of manganese in the oxygenated portion of the biosphere. Diverse bacteria and fungi have evolved the ability to catalyze this process, producing the manganese oxides found in soils and sediments. Other bacteria have evolved to utilize MnO_2 as a terminal electron acceptor in respiration. This Account summarizes the properties of Mn oxides produced by bacteria (bacteriogenic MnO_2) and our current thinking about the biochemical mechanisms of bacterial Mn(II) oxidation.

According to X-ray absorption spectroscopy and X-ray scattering studies, the MnO_2 produced by bacteria consists of stacked hexagonal sheets of MnO_6 octahedra, but these particles are extremely small and have numerous structural defects, particularly cation vacancies. The defects provide coordination sites for binding exogenous metal ions, which can be adsorbed to a high loading. As a result, bacterial production of MnO_2 influences the bioavailability of these metals in the natural environment. Because of its high surface area and oxidizing power, bacteriogenic MnO_2 efficiently degrades biologically recalcitrant organic molecules to lower-molecular-mass compounds, spurring interest in using these properties in the bioremediation of xenobiotic organic compounds. Finally, bacteriogenic MnO_2 is reduced to soluble Mn(II) rapidly in the presence of exogenous ligands or sunlight. It can therefore help to regulate the bioavailability of Mn(II), which is known to protect organisms from superoxide radicals and is required to assemble the water-splitting complex in photosynthetic organisms.

Bioinorganic chemists and microbiologists have long been interested in the biochemical mechanism of Mn(IV) oxide production. The reaction requires a two-electron oxidation of Mn(II), but genetic and biochemical evidence for several bacteria implicate multicopper oxidases (MCOs), which are only known to engage one-electron transfers from substrate to O_2. In experiments with the exosporium of a Mn(II)-oxidizing *Bacillus* species, we could trap the one-electron oxidation product, Mn(III), as a pyrophosphate complex in an oxygen-dependent reaction inhibited by azide, consistent with MCO catalysis. The Mn(III) pyrophosphate complex can further act as a substrate, reacting in the presence of the exosporium to produce Mn(IV) oxide. Although this process appears to be unprecedented in biology, it is reminiscent of the oxidation of Fe(II) to form Fe_2O_3 in the ferritin iron storage protein. However, it includes a critical additional step of Mn(III) oxidation or disproportionation. We shall continue to investigate this biochemically unique process with purified enzymes.

I. Introduction

Manganese(IV) oxide minerals are widely distributed in terrestrial and aquatic environments,[1] where they drive reactions fundamental to the ecological health of soils and natural waters. In particular, they adsorb metal ions avidly, playing key roles in the biogeochemical cycles of metals, including those of priority pollutants such as lead,

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thus stimulating interest in the development of Mn(IV) oxides for use in remediation applications.[2,3] This Account summarizes recent advances in understanding two important aspects of the chemistry of natural Mn(IV) oxides: their reactivity with metal cations and their formation by enzymatic mechanisms of Mn(II) oxidation.

The MnO_6 octahedra in most Mn(IV) oxide minerals assemble to form crystal structures of two principal kinds:[1,4] tunnel type, comprising chains of edge-sharing octahedra linked by shared corners into a three-dimensional framework permeated by rectangular tunnels (e.g., hollandite, todorokite), and layer type, comprising stacked sheets of edge-sharing octahedra, typically studded with defects (e.g., lithiophorite, birnessite). Tunnel-type Mn(IV) oxides, the variety found in oxic zones of Mn ore deposits and in marine nodules, may have provided the template for the oxygen-evolving center in photosystem II.[5] Layer-type Mn(IV) oxides, the variety abundant in soils, nodules, and rock varnishes as both colloids and grain coatings, are poorly crystalline (and thus highly reactive with metal cations), exhibiting structural disorder created by cation vacancies and random stacking arrangements.[1,6−10]

Oxidation of soluble Mn(II) is favorable thermodynamically but very slow.[11,12] At neutral pH, Mn(II) oxidation would require half a millennium[11] in the absence of catalysts or photochemical enhancement.[13] Morgan[12] has estimated the rates of homogeneous, heterogeneous (i.e., catalyzed by oxide mineral surfaces), and bacterial oxidation of soluble Mn(II) to be in the order 1:10:1000 under conditions representative of natural waters, leaving aside the possibility of photochemical influences.[11−15] Rate measurements in the field[15−17] likewise support the view that the Mn(IV) oxide minerals formed in ambient terrestrial and aquatic environments are precipitated directly by microbes[2,3] or by catalysis on the highly reactive biogenic Mn oxide surface.[18]

Bacteria and fungi that oxidize Mn(II) to Mn(IV) oxides are widespread in nature and phyllogenetically diverse,[3,19−22] but the physiological function of bacterial Mn(II) oxidation remains unknown. No correlation between CO_2 fixation and Mn(II) oxidation has been demonstrated, and other proposed biological functions of Mn(II) oxidation[3,23] (protection from toxic metals, reactive oxygen species, UV light, predation, or viruses; maintenance of an electron-acceptor reservoir for use in anaerobic respiration; breakdown of natural organic matter into metabolizable substrates; and scavenging of micronutrient trace metals) remain problematic.

Molecular genetics investigations to establish mechanisms of Mn(II) oxidation have focused on four phylogenetically distinct bacteria as model systems, *Bacillus* sp. strain SG-1,[24] *Pseudomonas putida*,[25] *Leptothrix discophora*,[26] and *Pedomicrobium* sp. ACM3067[27] for which sequence homology between the genes responsible for Mn(II) oxidation and those for the Cu(II) binding sites in multicopper oxidase (MCO) enzymes has recently been demonstrated.[22] The hypothesis that Mn oxidase enzymes are MCOs is supported by the fact that Cu(II) stimulates Mn(II) oxidation activity in the model bacteria,[3] but an apparent conundrum exists in reconciling the one-electron mechanism of MCOs with the two-electron oxidation transforming Mn(II) into Mn(IV).

II. Structure and Reactivity of Bacteriogenic Mn Oxides

Crystal Structure. Early studies of bacterial Mn(II) oxidation products using conventional X-ray diffraction (XRD) and electron microscopy identified the solid phases formed as Mn_3O_4 (hausmannite, which has a disordered spinel structure),[28,29] "buserite" (a hydrated layer-type Mn(IV) oxide),[1,30] γ-MnOOH (manganite, a tunnel-type Mn(III) oxide), and γ-MnO_2 (nsutite, a tunnel-type Mn(IV) oxide).[31] However, recent investigations[8−10,18,32−35] have convincingly disproven these tentative identifications. Except for "buserite", these candidate Mn solid phases, well-known as products of the abiotic oxidation of Mn(II), most likely resulted from autocatalytic oxidation of adsorbed Mn(II) on the surface of a freshly precipitated bacteriogenic Mn(IV) oxide.[18]

The Mn oxide mineral produced initially by the spore-forming marine *Bacillus* sp. strain SG-1 in 50 mM NaCl buffered near pH 8[9,10,18,32] was shown by *in situ* XRD to have hexagonal unit-cell symmetry. However, basal-plane XRD reflections were lacking, indicating either variable basal-plane spacing or unstacked single-layer material. Extended X-ray absorption fine structure (EXAFS) spectroscopy[9,10] also showed features characteristic of hexagonal Mn oxides but also indicated many Mn(IV) vacancies [up to 35% of the Mn(IV) sites, a value that derives in part from nanoparticle size].[9]

By comparison with chemically synthesized layer-type Mn(IV) oxides exhibiting hexagonal symmetry (hexagonal birnessites),[36−39] EXAFS spectroscopy indicates that each Mn ion in the bacteriogenic oxide structure is ideally di-μ-oxo bridged to six Mn neighbors with 2.82−2.90 Å Mn−Mn distances (Figure 1). In addition, 3.5−3.8 Å Mn−Mn distances are observed,[9,10] suggesting the presence of Mn^{3+} positioned above or below the Mn(IV) vacancy sites (Figure 2A).[37] More generally, the negative charge resulting from Mn(IV) vacancies can be compensated by the intercalation of hydrated

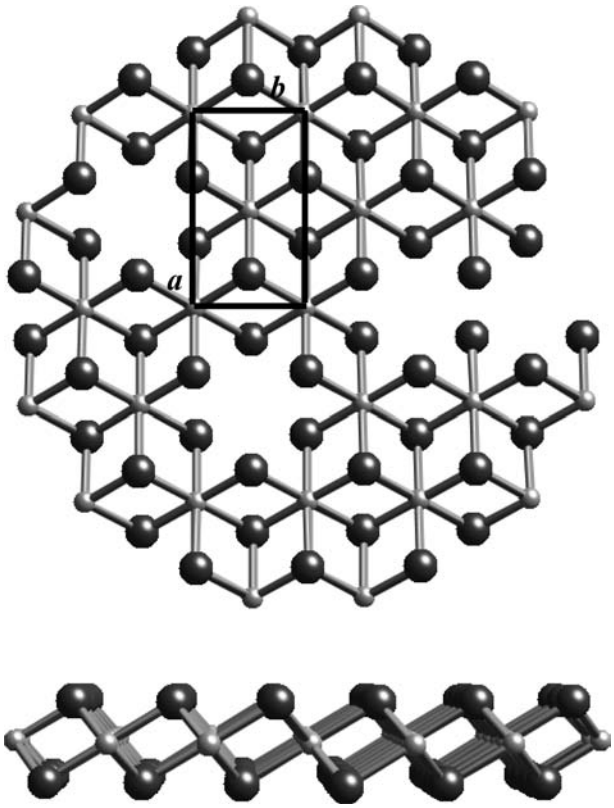

FIGURE 1. (A) Sheet structure in a hexagonal birnessite with 16 mol % Mn vacancies, highlighting a monoclinic unit cell. (B) Visualization of the sheet structure parallel to 010, illustrating di-μ-oxo bridging between neighboring Mn atoms.

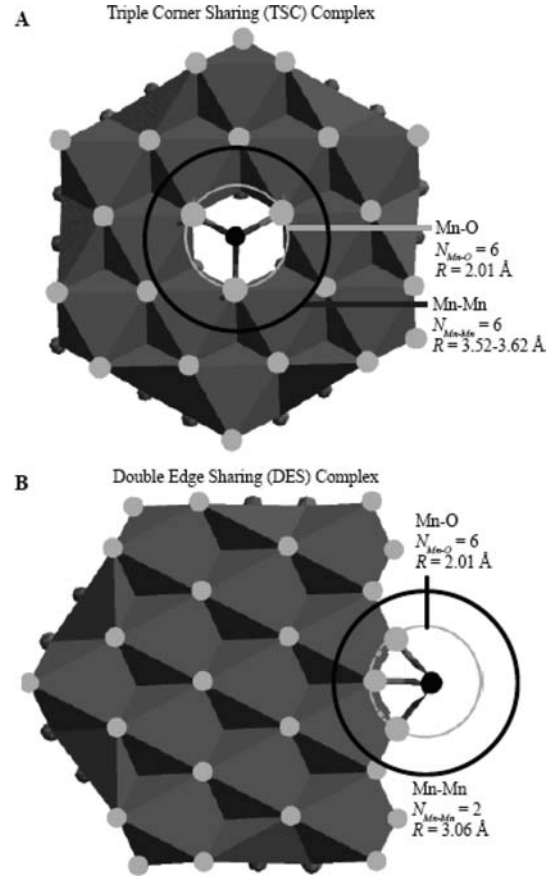

FIGURE 2. Visualization of adsorbed Pb(II) on hexagonal birnessite, showing (A) triple-corner-sharing and (B) double-edge-sharing inner-sphere surface complexes formed, respectively, above cation vacancy sites and at sheet edges.

metal cations,[1,8−10,18,32−39] resulting in basal plane spacings of 7−10 Å, depending on the extent of hydration.

In seawater, the initially formed bacteriogenic Mn(IV) oxide transforms to a secondary product having more stacked sheets and fewer cation vacancies [only up to 20% of the Mn(IV) sites].[9] X-ray absorption near-edge structure (XANES) spectra indicate reduction of about 20% of the Mn(IV) to Mn(III) during this structural transition, while the initial hexagonal symmetry is lowered, consistent with a partial ordering of the structure as a result of Mn(III) substitution.[36−39] Intriguingly, the charge-compensating intercalated cation is Ca^{2+}, not Mg^{2+}, even though Mg^{2+} is present in seawater at a 5-fold higher concentration than Ca^{2+}.

Well-defined XRD data for the Mn(IV) oxide produced by the soil and freshwater bacterium, *P. putida* strain MnB1/GB-1[8,35] were interpreted with a structural model having an average of 2.8 stacked sheets per particle and an 85 Å average *ab*-plane coherent X-ray scattering domain, with 17% of the Mn(IV) sites being vacant. An accompanying EXAFS analysis[8] yielded interatomic distances from which the local coordination environment above a Mn layer site vacancy can be visualized (Figure 2A). Similar results have been obtained by Jürgensen et al.[33] and Saratovsky et al.[34] for the Mn(IV) oxide precipitated by the sheath-forming freshwater bacterium *L. discophora* strain SP-6.

Field validation of the structural data obtained for laboratory-produced biogenic Mn oxides has come from analysis of stream bed sediments at Pinal Creek, Arizona, a well-characterized site of acid mine drainage contamination.[40,41] Microbial activity in the creek has produced 1−35 μm-thick Mn oxide coatings on silicate and Fe oxide minerals. Taken together, EXAFS, XRD, and TEM measurements[6] point to disordered pseudohexagonal layer-type Mn oxides with a 10 Å basal-plane spacing. These minerals are nanoparticulate (i.e., 11 nm thick × 35 nm in diameter) but with individual particles being as thin as a single sheet. More than 15% of the Mn(IV) sites are vacant and there is significant Mn(III) substitution, all of these characteristics being consistent with a microbial origin. Interestingly, Ca^{2+} is the charge-compensating intercalated cation, which is consistent with the high affinity for Ca^{2+} observed in the case of the Mn oxide formed in seawater.[9]

Villalobos et al.[8,35] have characterized two poorly crystalline, chemically synthesized analogs of bacteriogenic Mn oxides. Like the bacteriogenic Mn oxides, these analogs exhibit abundant vacancies, stacking disorder, and nanoparticle size (e.g., basal-plane spacings of 19−42 Å and coherent X-ray scattering domains of 60−70 Å in the *ab* plane). A well-crystallized synthetic analog also has been prepared and characterized by Gaillot et al.[37]

Reactivity with Metal Cations. A long history of field and laboratory evidence pointing to strong metal cation adsorption by Mn oxides[2,3] has stimulated numerous recent studies to elucidate mechanisms.[42−51] Key to understanding these mechanisms are the structural O ions with uncompensated bond valences that are found surrounding Mn(IV) vacancy sites (Figure 1A) and at sheet edges.[43,44]

The well-known high-affinity association of Pb(II) with Mn(IV) oxides found in contaminated settings[3] motivated Villalobos et al.[51]and Takahashi et al.[48] to perform EXAFS spectral analyses of Pb(II) adsorbed by both laboratory-produced and naturally occurring Mn(IV) oxides. The dominant Pb−Mn distances were 3.7 Å and 5.56 Å, consistent with Pb(II) bound in triple-corner-sharing inner-sphere surface complexes (Figure 2A), but an additional 3.2 Å Pb−Mn distance led Takahashi et al.[48] to include a double-edge-sharing Pb(II) inner-sphere surface complex at particle edges (Figure 2B). Significant Pb(II) retention on particle edge surfaces is supported by the strong positive correlation observed between the adsorbed Pb/Mn molar ratio and specific surface area for both bacteriogenic birnessites and their synthetic analogs.[51]

Like Pb(II), Zn(II) speciation and mobility in soils and sediments often is observed to be controlled by Mn(IV) oxides, motivating Toner et al.[50] to perform an EXAFS spectral analysis of Zn adsorbed on the Mn oxide produced by *P. putida*. In their experiments, the bacteriogenic oxide mineral was embedded within the biofilm this bacterium normally inhabits. At adsorbed Zn/Mn molar ratios below 0.2, Zn(II) was found to be in tetrahedral coordination with one water O and three O ions surrounding a Mn(IV) vacancy site, as has been inferred also for Zn adsorbed at low levels by other natural and synthetic birnessites.[42−44,50] As the Zn/Mn molar ratio increased, however, a shift to octahedral coordination was indicated, with the surface complex now involving three water O and three O ions surrounding a vacancy site. Significantly, the organic material in the hydrated biofilm did not intervene in Zn adsorption until all Mn(IV) vacancy sites in the oxide were capped by Zn. This important result, which has also been reported for Pb(II) on biogenic Mn oxides,[52,53] indicates that bacterial biofilms do not tend to mask trace metal adsorption by the embedded biogenic Mn oxides.

III. Mechanisms of Bacterial Mn(II) Oxidation

Oxidation of Mn(II) to Mn(III). Three model bacteria, *Bacillus* sp. strain SG-1,[25]*Pseudomonas putida* strains MnB1 and GB-1,[24] and *Pedomicrobium* sp. ACM3067,[27,54] have been shown to require a MCO in the oxidation of Mn(II) to MnO_2. Loss of the ability to oxidize Mn(II) is associated with disruption of a gene (MnxG, CumA, and MoxA, respectively) whose sequence identified it as an MCO; all of the diagnostic Cu-binding residues are present (Figure 3). Cell homogenates from these and related organisms display protein bands on polyacrylamide gels that produce a brown solid precipitate upon exposure to Mn(II).[55−57] Sequence analysis of nine distinct peptides confirm that the protein associated with the Mn oxide bands from *Bacillus* spp. strains PL-12 and MB-7 is indeed the MCO MnxG.[58] In a fourth model bacterium, *Leptothrix discophora* strain SS-1, a Mn(II)-oxidizing protein band is observed[26,59] and antibodies raised to such a band were found to cross-react with a MCO gene (MofA) product produced in an expression library.[26]

Multicopper oxidases are one-electron oxidants in which electrons are transferred singly from the substrate to O_2 through intervening Cu ions.[60] Therefore, if Mn(II) is oxidized by a bacterial MCO, the initial product expected is Mn(III), not Mn(IV). To test this hypothesis, Webb et al.[61] added the strong Mn(III)-complexing ligand pyrophosphate (PP) to preparations of exosporium (the loose outer part of the spore coat where MnO_2 is formed) from *Bacillus* sp. SG-1. In the presence of PP, a steady growth in the absorption band at 258 nm, characteristic of the Mn(III)−PP complex, was observed (Figure 4), establishing that the spores do indeed oxidize Mn(II) in a one-electron step. Moreover, Mn(III) formation was inhibited by the addition of azide, a well-known MCO inhibitor. Growth of the signature absorption band at 258 nm was accelerated by adding Cu(II), suggesting that some of the Cu sites in MnxG are labile.

Oxidation of Mn(III) to MnO_2. Webb et al.[61] also found that the 258-nm Mn(III)-PP absorption band reached a maximum and then declined over time (Figure 4) while brown solid particles were observed, implying oxidation of Mn(III) to form the solid phase MnO_2. When Mn(III)-PP was added to the exosporium preparation in the absence of Mn(II), the 258-nm absorption band decreased at the same rate as it did subsequent to Mn(II) oxidation, whereas in the absence of exospo-

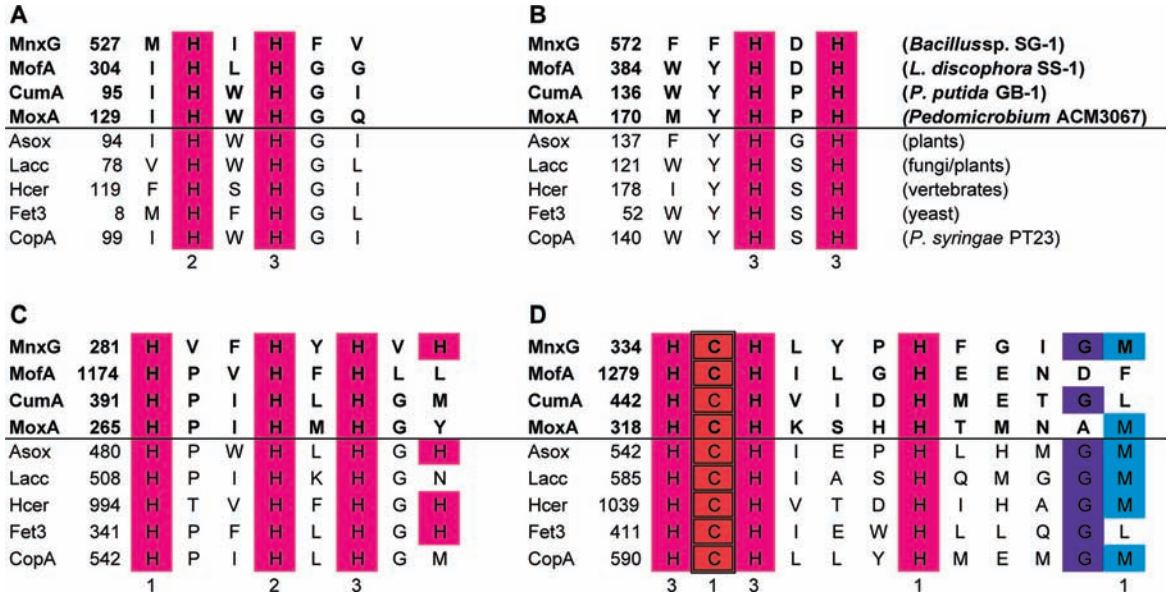

FIGURE 3. Amino acid sequence alignment of two of the conserved Cu binding regions of multicopper oxidases from four model Mn(II)-oxidizing bacteria (Tebo et al.[2]).

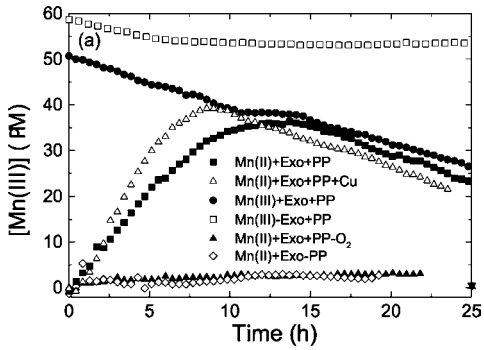

FIGURE 4. Evolution of Mn(III) as a function of time following addition of Mn(II) to exosporium of *Bacillus* sp. strain SG-1 as determined by trapping with pyrophosphate ligands (Webb et al.[61]).

rium, the signal was undiminished over the same time interval. A mutant strain deficient in MnxG was shown to be unable to oxidize either Mn(II) to Mn(III) or Mn(III) to Mn(IV). Therefore, MnxG must catalyze not only the initial oxidation of Mn(II) to Mn(III) but also the oxidation of Mn(III) to MnO_2.

It is improbable that the MCO extracts a second electron from the intermediate Mn(III) to produce mononuclear Mn(IV). The latter is a powerful oxidant and cannot be stabilized by the relatively weak donor ligands available from protein side chains, which are likely to be carboxylate groups. In the case of the mammalian MCO ceruloplasmin, which oxidizes Fe(II) to Fe(III), Lindley et al.[62] found that Fe(II) binds to a site adjacent to the type 1 Cu and, after electron transfer, Fe(III) moves several angstroms toward the solvent interface to a "holding site". The donor ligands at both sites are carboxylates derived from aspartate and glutamate residues. In the exosporium experiments of Webb et al.,[61] a similar translocation in the putative *Bacillus* MCO, after the initial electron extraction from Mn(II), could provide a natural pathway for the facile movement of Mn(III) to exogenous PP.

Most known Mn(IV) complexes are polynuclear and stabilized by oxide bridges (e.g., Pizarro et al.[63]). In the absence of capping ligands, the Mn(IV) clusters polymerize further to form solid-phase MnO_2. If the "holding site" in a bacterial MCO can accommodate multiple Mn(III) ions, it might then stabilize a polynuclear Mn(IV) complex as a nucleation site for MnO_2 nanoparticle formation. The first step could be further oxidation of Mn(III), either via the MCO or possibly by direct reaction with O_2, to form a polynuclear oxo-bridged Mn(IV). Alternatively the multiple Mn(III) ions could disproportionate[11] to a polynuclear Mn(IV) complex and Mn(II) ions, which would then be promptly reoxidized at the MCO substrate site, ensuring a continuous supply of Mn(III). In either case, the polynuclear Mn(IV) complex formed at the nucleation site would be released ultimately to grow into MnO_2 nanoparticles.

A possible scheme (Figure 5) involves a $[Mn(IV)_2O_2]^{4+}$ core at an appropriate binuclear binding site. Such sites are common in metalloproteins, a pertinent example being the binuclear Mn catalase enzymes,[64] in which a pair of Mn ions cycles between the Mn(II) and Mn(III) oxidation states during enzymatic turnover. Another useful example is the ferroxidase site in the iron storage protein, ferritin,[65] wherein a pair of Fe(II) ions is oxidized to Fe(III) en route to the accumulation of ferric oxyhydroxide nanoparticles in the protein interior. Ferritin might well serve as a useful prototype for thinking about bacterial MnO_2 formation, but with the critical additional

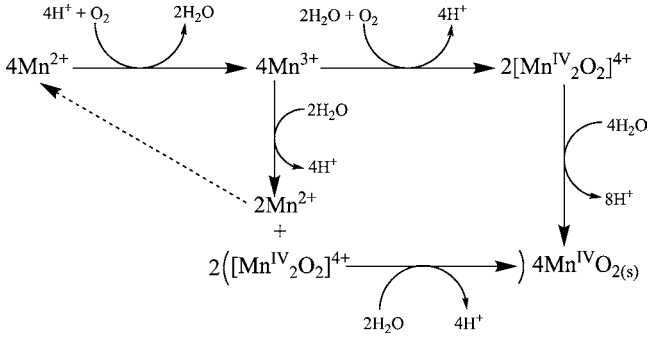

FIGURE 5. Proposed scheme for bacterial MnO_2 formation by multicopper oxidase-catalyzed Mn^{2+} oxidation, followed by further oxidation (top) or by disproportionation of complexed Mn^{3+} (down arrow).

step of Mn(III) oxidation or disproportionation. However, since the Mn(III)-PP experiments of Webb et al.[61] were carried out on bacterial exosporium, not on isolated protein, it is possible that MnO_2 formation could occur on a protein other than the putative MCO. If this alternative hypothesis were true, Mn(III) would have to migrate between two sites using a carrier molecule to prevent disproportionation.

IV. Concluding Remarks

That bacteriogenic Mn oxides can strongly bind metal cations possessing coordination geometries as diverse as Zn(II) (octahedral and tetrahedral coordination), Pb(II) (distorted trigonal pyramidal with stereoactive electron lone pair), and U(VI) (hexagonal bipyramidal) underscores their exceptional qualities as adsorbents.[3] The use of bacteriogenic Mn oxides for the engineered removal of toxic metals from wastewaters and contaminated soils has long been proposed,[3,47,52] and therefore, Mn(II) oxidation to MnO_2 has potential for the engineered immobilization of dissolved metals in contaminated aquifers or hyporheic zones in which microaerophilic or oxic conditions prevail.[6,66] Manganese oxides have long played major technological roles in the manufacture of catalysts, colorants, metal sorbents, and batteries (e.g., see the review by Saratovsky et al.[34]), suggesting that the excellent adsorbent properties of bacteriogenic Mn oxides offer the possibility to enhance these technological applications significantly.

Bacterial Mn(II) oxidation is one of the major processes by which Mn(II) is exported from the marine euphotic zone to underlying ocean waters.[15] Manganese oxides settling through the oceanic water column provide the reactive surface area that scavenges trace metal nutrients such as Co(III) and possibly Fe(III).[67] From this Account, we may conclude that these minerals are nanoparticulate layer-type Ca-bearing Mn oxides. Intriguingly, the concentration of Mn(II) in the surface mixed layer is orders of magnitude higher (0.5−1 nM[11,68]) than that expected from Mn(II) in equilibrium with birnessite in seawater (<10^{-14} M[30]), probably because of photoinduced reductive dissolution[11,15] and the slowness of Mn(II) reoxidation. Recent experimental and theoretical work[69,70] suggests that, because of its vacancy defects, nanoparticulate hexagonal birnessite is particularly susceptible to photoreduction, and it is likely that the small particle size enhances the photoreduction rate.

The finding that bacteria produce labile Mn(III)[61] raises new and intriguing questions about the environmental role of this transient Mn oxidation state, long presumed to be of limited significance in natural settings.[71] For example, recent work by Trouwborst et al.[72] in the Black Sea demonstrated that soluble Mn(III) may contribute up to 100% of the dissolved Mn in the suboxic zone, and it is possible that Mn(II)-oxidizing bacteria are a significant source of this strong oxidant in other environments.[71] When solubilized by robust chelators, such as siderophores,[73,74] Mn(III) can be utilized by microorganisms to metabolize recalcitrant organic substrates such as lignin, to accept electrons during anaerobic growth, or to protect against toxic oxygen species.

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John Bargar, senior research scientist at the Stanford Synchrotron Radiation Laboratory, received his B.S. in Geology and Mineralogy (1990) from The Ohio State University and his Ph.D. in Geological and Environmental Sciences from Stanford University (1996). Bargar's principal research interests lie in the areas of geomicrobiology, low-temperature aqueous geochemistry, and mineral−water interface geochemistry. His current research activities focus on the structural chemistry and environmental reactivity of bacteriogenic minerals, investigated under in situ conditions with synchrotron-based scattering and

spectroscopy techniques, with emphasis on elucidating their roles in the biogeochemical cycling of elements in the biosphere.

Garrison Sposito is a professor in the Department of Environmental Science, Policy and Management in the University of California at Berkeley. He received B.Sci. and M.Sci. degrees from the University of Arizona and a Ph.D. from the University of California at Berkeley. His research interests include the surface chemistry of natural nanoparticles, molecular simulations of nanoparticle structure and reactivity, and the chemistry of humic substances. He has been elected a Fellow of six scientific societies and is a recipient of Fulbright, Guggenheim, and NATO-Heinemann fellowships. In 2008, he was among 15 chemists designated as a "Legend in Environmental Chemistry" by the American Chemical Society.

Bradley Tebo is a Professor in the Division of Environmental and Biomolecular Systems, Oregon Health & Science University. He received his B.A. in Biology from UCSD and his Ph.D. in Marine Biology from Scripps Institution of Oceanography (UCSD). After his postdoctoral work in the School of Oceanography at the University of Washington and a brief period as a Research Scientist at the Chesapeake Bay Institute, Johns Hopkins University, he became a Research Scientist and subsequently Professor-in-Residence at Scripps Institution of Oceanography for 18 years until moving to his present position in 2005. He is a Fellow of the American Academy of Microbiology. His research focuses on the geomicrobiology of metal transformations, spending most of his career studying the microbiology and biogeochemistry of bacterial Mn(II) oxidation.

FOOTNOTES

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