Heterologous Expression and Characterization of the Manganese-Oxidizing Protein from *Erythrobacter* sp. Strain SD21

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The manganese (Mn)-oxidizing protein (MopA) from *Erythrobacter* sp. strain SD21 is part of a unique enzymatic family that is capable of oxidizing soluble Mn(II). This enzyme contains two domains, an animal heme peroxidase domain, which contains the catalytic site, followed by a C-terminal calcium binding domain. Different from the bacterial Mn-oxidizing multicopper oxidase enzymes, little is known about MopA. To gain a better understanding of MopA and its role in Mn(II) oxidation, the 238-kDa full-length protein and a 105-kDa truncated protein containing only the animal heme peroxidase domain were cloned and heterologously expressed in *Escherichia coli*. Despite having sequence similarity to a peroxidase, hydrogen peroxide did not stimulate activity, nor was activity significantly decreased in the presence of catalase. Both pyrroloquinoline quinone (PQQ) and hemin increased Mn-oxidizing activity, and calcium was required. The K_m for Mn(II) of the full-length protein in cell extract was similar to that of the natively expressed protein, but the K_m value for the truncated protein in cell extract was approximately 6-fold higher than that of the full-length protein, suggesting that the calcium binding domain may aid in binding Mn(II). Characterization of the heterologously expressed MopA has provided additional insight into the mechanism of bacterial Mn(II) oxidation, which will aid in understanding the role of MopA and Mn oxidation in bioremediation and biogeochemical cycling.

Manganese (Mn)-oxidizing bacteria and fungi are known to play an important role in the biogeochemical cycling of Mn and other elements by catalyzing the oxidation of soluble Mn(II) to Mn(III, IV) oxides. The Mn oxides formed can oxidize reduced metals, sulfides, and carbon compounds. In addition, the amorphous structure of the biogenic oxides can sequester heavy metals, reducing their toxicity (1–4). Mn-oxidizing bacteria have the potential to increase the rate of Mn oxidation by orders of magnitude over abiotic Mn oxidation, and this capability is found in many diverse phylogenetic groups (4). Yet, understanding the enzymes involved in Mn oxidation by these diverse bacteria has been difficult to achieve due to low levels of native expression and often unstable activity, which has prevented the purification and investigation of the enzymes under native conditions (5–7).

Multicopper oxidase (MCO) enzymes have been implicated in several of the model Mn-oxidizing strains (8). MCOs use molecular oxygen as the oxidant, with electrons being transferred from the substrate to molecular oxygen via multiple copper atoms in the enzyme. These enzymes are capable of oxidizing a broad range of substrates, including organic compounds, phenolic compounds, and various metals, such as Fe(II) and Mn(II) (9). They have been identified in *Pseudomonas putida* (10), *Bacillus* species (11, 12), *Leptothrix discophora* (13), and *Pedomicrobium* (14). Recently, two of the bacterial Mn-oxidizing MCO enzymes from *Bacillus* species (15, 16) and an MCO, CueO, from *Escherichia coli* (17) have been heterologously expressed, purified, and biochemically characterized. These unique MCO enzymes oxidize Mn(II) by two one-electron transfers to form an Mn(IV) oxide. The CotA protein from *Bacillus pumilus* WH4 and the CueO from *E. coli* can catalyze Mn(II) oxidation with a single subunit, yet the MnxG multicopper oxidase from *Bacillus* sp. strain PL-12 requires coexpression of the accessory proteins MnxDEF for activity (16).

In addition to the MCOs, another class of bacterial Mn-oxidizing proteins has been described. These proteins have sequence similarity to animal heme peroxidases of the peroxidase-cyclooxygenase superfamily of peroxidases (18). This class of enzymes includes myeloperoxidases (MPO) and lactoperoxidases (LPO), which use hydrogen peroxide as the oxidant and, as their names suggest, contain a heme cofactor. These animal heme peroxidases generally consist of the peroxidase domain and another nonenzymatic domain (18, 19). The Mn-oxidizing proteins (MopAs) of this type have been identified in *Erythrobacter* sp. strain SD21 and *Aurantimonas manganoxydans* strain SI85-9A1 by protein sequencing of Mn-oxidizing SDS-PAGE bands (5). These Mn-oxidizing proteins are in the peroxicin subfamily of the peroxidase-cyclooxygenase superfamily (18, 20). This subfamily has one or more animal heme peroxidase domains, with an additional domain containing multiple RTX-type calcium binding motifs (Fig. 1). Several proteins in this family, including the Mn-oxidizing protein from *A. manganoxydans* SI85-9A1, have repeated domains (18). RTX proteins are found in many Gram-negative bacteria and contain glycine and aspartate-rich nonapeptide repetitions for the binding of calcium ions (21). Although the functions of many animal heme peroxidases and RTX proteins are unknown, there are a variety of known functions for both of these types of proteins, including antibacterial activity for many animal heme peroxidases and protease activity and S-layer formation (21) for RTX-type proteins. The Mn-oxidizing proteins of this type oxidize Mn(II) to Mn(III) (5, 6). The Mn(III) formed from these organisms may form Mn(IV) oxides via another enzyme, such as an MCO, or by disproportionation. Mn(III) may also remain as Mn(III) bound to a ligand, as it has recently been identified in the environment (8, 22). The partially purified MopA protein from

FIG 1 Protein diagram of the manganese-oxidizing peroxidase/protein (MopA). MopA, identified in *Aurantimonas manganoxydans* strain SI85-9A1 and *Erythrobacter* sp. strain SD21, has an animal heme peroxidase domain(s) and an RTX calcium binding domain.

Aurantimonas manganoxydans SI85-9A1 has been shown to contain a heme cofactor via heme staining of an SDS-PAGE gel and to have Mn-oxidizing activity stimulated by hydrogen peroxide (5). *In vitro* studies on the Mn-oxidizing protein from *Erythrobacter* sp. SD21 did not show stimulation in the presence of hydrogen peroxide, but this activity was stimulated by PQQ and NAD$^+$/NADH, and PQQ was found to copurify with the partially purified Mn(II)-oxidizing protein (6). The activity in native cell extract was also inhibited by peroxidase inhibitors cyanide, azide, and phenylhydrazine (6). The inability to purify the native active enzymes from these strains has significantly hindered their characterization and further understanding of this unique bacterial process.

Mn-oxidizing bacteria and their enzymes play an important role in the environment. Both the manganese-oxidizing peroxidase (Mop) type and the MCOs have been identified in Mn-oxidizing environmental samples (23), suggesting that both types of enzymes are likely to be relevant *in situ*. Although recent studies on MCOs have started to reveal the mechanism of this reaction, little is known about Mop enzymes. To better understand these newly identified animal heme peroxidase-type bacterial Mn-oxidizing enzymes, we have cloned the gene encoding MopA and the animal heme peroxidase (AHP) domain of MopA from *Erythrobacter* sp. SD21 and heterologously expressed this enzyme in *E. coli*. Characterization of the heterologously expressed enzyme begins to tell the story of how this unique enzyme may function in Mn oxidation.

MATERIALS AND METHODS

Cloning of the manganese(II)-oxidizing protein. *Erythrobacter* sp. SD21 genomic DNA was isolated using standard techniques, and the *mopA* gene was amplified with *Pfu* Turbo (Agilent) using the forward primer 5′-AT GAAAAAACTGCTGTTCGCGATTCCGCTGGTGGTGCCGTTCTATA GCCATAGCATGGCCGTCAAACTCAACAAGC-3′, which contains a gIII signal sequence, and the reverse primer 5′-ATGATGATGATGATGA TGGTCGACGGCGCTATTGAAGAGGAAGGTTTCG AAGAGTCC-3′, encoding a histidine tag. The 6.4-kb amplicon was cloned into the pSpeedET vector using the polymerase incomplete primer extension (PIPE) method (24). The pSpeedET vector used contains an inducible arabinose promoter for overexpression, a histidine tag for purification, and a kanamycin resistance gene for selection. The amplified vector used for PIPE cloning of the full-length *mopA* gene did not include the N-terminal histidine tag but had an added C-terminal histidine tag. The pSpeedET-Mop construct was designed for a 6× C-terminal histidine tag, but sequencing of different clones revealed some of the pSpeedET-Mop constructs had 12× and 18× histidine tags, thus allowing us to see if the histidine tag length could affect activity and purification.

A truncated version of the *mopA* gene encoding the catalytic AHP domain (nucleotides [nt] 1 to 2787) was cloned into the same pSpeedET expression vector by using PIPE cloning with the standard N-terminal 6×His tag. The AHP domain of *mopA* was amplified with the forward primer 5′-CTGTACTTCCAGGGCATGGCCGTCAAACTCAACAAG C-3′ and the reverse primer 5′-AATTAAGTCGCGTTAATTCAGCACTT CGTCATCGTGTTGC-3′. The AHP domain of *mopA* was also cloned into the pBAD/gIII expression vector (Life Technologies) by PIPES cloning. The vector was amplified using 5′-GAACAAAAACTCATCTCAGAA GAGGATCTGAATAGGCCG-3′ and 5′-GGTGCTATGGCTATAGAAC GGCACCACCAGCGGAATCGCGAACCAGC-3′. The AHP domain of *mopA* was amplified for cloning into the pBAD/gIII vector using 5′-TTC TATAGCCATAGCACCATGGCCGTCAAACTCAACAAGC-3′ and 5′-GATGAGTTTTTGTTCATTCAGCACTTCGTCATCGTGTTGC-3′. All plasmids were cloned into chemically competent *E. coli* HK100 (24) for purification and sequencing. The resulting pSpeedET-Mop, pSpeedET-AHP, and pBAD/gIII-AHP recombinant plasmids were transformed into chemically competent *E. coli* Rosetta 2 (Novagen) cells carrying the pRARE plasmid that encodes rare tRNAs and contains a chloramphenicol resistance gene.

Overexpression of Mop and preparation of cell extract. *E. coli* Rosetta 2 cells transformed with the pSpeedET-Mop and the pSpeedET-AHP plasmid were inoculated into LB medium supplemented with 60 μg/ml kanamycin and 30 μg/ml chloramphenicol and incubated overnight at 37°C and 250 rpm. A total of 3 ml of the overnight culture was added to 100 ml of LB medium with the same antibiotics under the same conditions. Once the cultures reached an optical density at 600 nm (OD$_{600}$) of ~0.5, they were induced with 0.02% L-arabinose and incubated for 4 h. The harvested cells were resuspended in 50 mM HEPES, pH 7.7, and lysed with a French press (16,000 lb/in^2). The soluble cell extract was obtained by centrifugation at 9,100 × g for 20 min at 4°C. SDS-PAGE (25) and Western blotting were used to confirm expression. Western blots using the HisProbe-horseradish peroxidase (HRP) and SuperSignal West His-Probe kit (Thermo Scientific) were used according to the manufacturer's suggestions.

Mn oxidation assay. Mn-oxidizing activity was determined as previously described (6). A 500-μl assay contained approximately 1.7 mg cell extract, 1 mM MnCl$_2$, 10 mM CaCl$_2$, 10 μM PQQ, 87.6 mM NaCl, and 43.8 mM HEPES (pH 8). The assay was incubated for 24 h with shaking (250 rpm). All assays were incubated at room temperature, similar to the optimal growth temperature for *Erythrobacter* sp. SD21 (26). Product formation increased linearly with time for more than 24 h, as determined by a time course. Catalase (5 μM), superoxide dismutase (50 μM), and hemin (0.5 μM) were added, as indicated. All assays were performed in triplicate. Oxidized Mn [Mn(III) or Mn(IV)] was quantified by adding 50 μl of the assay to 250 μl of 0.014% or 0.04% leucoberbelin blue (3), incubating for 15 min at room temperature, centrifuging (20,000 × g, 5 min, room temperature) to remove precipitated protein, and determining the A$_{620}$. Potassium permanganate served as the standard.

For determination of kinetic parameters, Mn(II) was added at 0 mM, 0.1 mM, 0.4 mM, 0.6 mM, 1 mM, 1.5 mM, 2.0 mM, 5.0 mM, and 10 mM. Oxidized Mn was measured every 1 to 2 h for approximately 12 h to determine the rate of oxidation. K_m and V_{max} values were determined with the graphing software Kaleidograph (Synergy Software) fitted to the Michaelis-Menten kinetic equation.

Protein quantification, chemicals, and reagents. Total protein was determined using the Coomassie Plus assay reagent (Pierce) with bovine serum albumin as the standard. All chemicals were reagent grade from Fisher or Sigma-Aldrich.

RESULTS AND DISCUSSION

Heterologously expressed MopA and the AHP domain catalyze Mn(II) oxidation. In order to increase expression of and aid in purification of the Mn-oxidizing protein as well as to determine the domain necessary for activity, *mopA* and the AHP domain of

mopA (Fig. 1) were cloned and heterologously expressed in *E. coli* Rosetta 2. The operon containing the *mopA* gene in *Erythrobacter* sp. SD21 has not been characterized, and *mopA* may be the only gene in its operon. Upstream of *mopA*, there is a two-component system response regulator that has currently not been linked to Mn oxidation. Therefore, no additional genes were expressed with *mopA*. The full-length *mopA* gene was cloned into the modified pSpeedET expression vector carrying a C-terminal His tag and a gIII N-terminal signal sequence. Several constructs with the His tag on the N or C terminus, different lengths of the His tag, and different expression vectors were tested for activity, with the most stable Mn-oxidizing activity occurring when expressed with an 18× C-terminal His tag and the N-terminal gIII signal sequence in the pSpeedET vector. The AHP domain was cloned into the pSpeedET expression vector and expressed with an N-terminal His tag. It was also cloned into the pBAD/gIII vector with a C-terminal His tag and an N-terminal gIII signal sequence. Activity and expression were relatively similar for the two constructs (0.184 nmol · min^{-1} · mg cell extract^{-1} for pSpeedET expression and 0.082 nmol · min^{-1} · mg cell extract^{-1} for pBAD/gIII); thus, the N-terminal His-tagged protein expressed from the pSpeedET vector was used for further studies. For both the AHP domain and the full-length MopA, the presence of the gIII signal did not help to purify an active protein from the periplasm.

An active MopA was expressed in *E. coli* cell extract, indicating that MopA does indeed catalyze Mn(II) oxidation. *E. coli* cell extract is unable to oxidize Mn in the absence of these expressed proteins, and assays containing heat-denatured protein or no protein did not oxidize Mn. The Mn-oxidizing activity of these heterologously expressed proteins strongly builds on their identification in Mn-oxidizing SDS-PAGE gels (5). The full-length 225-kDa MopA was expressed at a level comparable to expression in the native host (27) and ran as two bands at 150 and 250 kDa. These two bands have previously been seen with the native system (27). Affinity, ion exchange, gel filtration, and hydrophobic interaction chromatography were performed using a variety of conditions to purify the heterologously expressed enzyme, but Mn-oxidizing activity was lost. In addition, *E. coli* proteins that may stabilize, interact with, or stimulate Mn-oxidizing activity were investigated, but adding back *E. coli* cell extract did not rescue the lost Mn-oxidizing activity, nor did the addition of heme or pyrroloquinoline quinone (PQQ) (data not shown). Thus, a purified, active Mn-oxidizing enzyme could not be obtained, a problem previously encountered with purification from the native host (6). Therefore, the heterologously expressed enzyme was studied in cell extract.

The AHP domain was also actively expressed in *E. coli*. As indicated in Fig. 2, the 105-kDa AHP domain is expressed at much higher levels than the full-length protein. Similar to the full-length MopA, it does not run true to size on SDS-PAGE. The activity of the AHP domain alone suggests it is the catalytic domain, as has been found in other animal heme peroxidases with additional domains (19). This finding is intriguing in this case, since the calcium binding domain is found in concert with the AHP domain in both *Erythrobacter* sp. SD21 and *Aurantimonas manganoxydans* SI85-9AI and in several other organisms. Mn-oxidizing *Pseudomonas putida* GB-1 contains an MopA protein containing two AHP domains with calcium binding domains similar to those of *A. manganoxydans* SI85-9A1, although this protein has not been implicated in Mn oxidation (10). *Leptothrix cholodnii* SP6, another
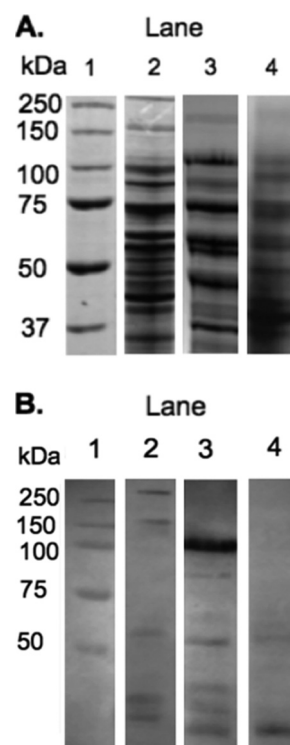
FIG 2 Heterologous expression of MopA and the AHP domain in *E. coli* cell extract: SDS-PAGE stained with Coomassie blue (A) and Western blot of the expression of the full-length MopA and the AHP domain from MopA (B) in *E. coli* cell extract. Lane 1, protein standard; lane 2, expression of the full-length protein; lane 3, expression of the AHP domain; lane 4, *E. coli* cell extract. Lanes shown were extracted from nonadjacent lanes or different gels and aligned using the protein standard.

Mn-oxidizing strain, also has an animal heme peroxidase protein with a calcium binding domain (28), but the role of this protein in Mn oxidation is not known at this time. MopA-like proteins (with AHP and calcium binding domains) are not unique to known Mn oxidizers, as they can also be found in the genome sequences of *Rhodopseudomonas palustris* BisB5, *Methylobacterium chloromethanicum* CM4, and other species that have not been implicated in Mn oxidation.

H$_2$O$_2$ is not likely a substrate for Mn-oxidizing activity of MopA. Because of the presence of the AHP domain and the sequence similarity to enzymes known to use hydrogen peroxide as a substrate, the effect of hydrogen peroxide was determined on enzyme activity for both the full-length enzyme and the AHP domain. Hydrogen peroxide (1 to 1,000 μM) was added to the enzyme assay, and there was no stimulatory effect on activity. In fact, high levels of hydrogen peroxide (>50 μM) decreased activity, likely due to reducing the oxidized Mn formed in the reaction (29). Catalase is likely present in the *E. coli* cell extract (30), but it may not be highly active, since addition of hydrogen peroxide decreased Mn oxidation. Therefore, additional catalase was also added to the enzyme assay, as it should inhibit any reactions requiring hydrogen peroxide. As shown in Fig. 3, catalase had only a small effect on Mn-oxidizing activity, providing further evidence that hydrogen peroxide is not likely a substrate. Studies on MPO indicate that hydrogen peroxide interacts with the histidine distal to the heme and an arginine at the catalytic site (31). Both residues
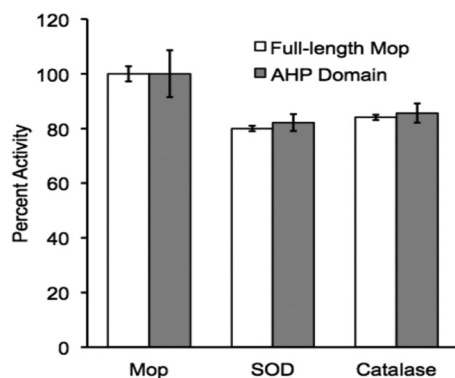
FIG 3 Superoxide and hydrogen peroxide are not substrates for MopA. The addition of 50 μM superoxide dismutase (SOD) and 5 μM catalase did not abolish Mn(II) oxidation of the full-length and the AHP domain in cell extract. Percent activity is compared to activity in the absence of SOD and catalase. Error bars represent standard deviations from triplicates.

TABLE 1 Effect of PQQ, hemin, and calcium on Mn oxidation

Protein or domain	Component	% activity ± SD[a]
MopA from native host	PQQ	824 ± 10 (6)
Full-length MopA	PQQ	150 ± 0.5
AHP domain	PQQ	160 ± 0.8
Full-length MopA	Hemin	108 ± 2.6
AHP domain	Hemin	136 ± 6.8
Mop from native host	Calcium	140 ± 10 (6)
Full-length MopA	Calcium	Required
AHP domain	Calcium	Required

[a] Values are from triplicates.

are conserved in MopA. If H_2O_2 is a substrate of the Mn oxidation reaction, it may be difficult to determine, as the presence of H_2O_2 can affect the abundance of the product (H_2O_2 reacts directly with Mn oxides), but it does not appear to be required.

Recently, Mn oxidation in *Roseobacter* sp. strain AzwK-3b was found to occur as a result of enzymatic superoxide formation (32). To assess if superoxide may be a substrate for MopA or a product of MopA that then catalyzes Mn oxidation, superoxide dismutase (SOD) was added to the assay (Fig. 3). SOD is also likely present in *E. coli* cell extract (33), which would prevent the accumulation of superoxide; additional SOD was added to be certain no superoxide was present in the assay and potentially oxidizing Mn(II). SOD addition did cause a slight decrease in activity, but not to the extent one would expect if superoxide acted as a substrate for the Mn oxidation reaction.

The natively expressed protein was inactive in the absence of oxygen (H. A. Johnson and B. M. Tebo, data not shown), suggesting that oxygen may be the terminal electron acceptor for MopA-catalyzed Mn oxidation. Coupling Mn oxidation to oxygen, or any other compound except hydrogen peroxide, seems very unusual for a protein with significant sequence similarity to peroxidases. MopA may oxidize Mn using a very novel mechanism. A purified protein will be required to further probe the mechanism of action.

Heme and PQQ stimulate MopA Mn oxidation. Animal heme peroxidases are known to require a heme prosthetic group. This heme group is covalently attached to aspartate and glutamate residues in LPO (34) and to an additional methionine in MPO (31). The heme is also hydrogen bonded to a proximal histidine which interacts with an essential asparagine (35, 36). The aspartate and glutamate residues binding the heme are conserved among animal heme peroxidases and are present in MopA. For both the full-length and the truncated protein in cell extract, there is an increase in activity with the addition of 0.5 μM hemin (see Table 2). This increase in activity suggests that heme is important for activity. It also suggests that heme may be limited during expression, but addition of hemin (770 μM) or the heme precursor, 5-aminolevulinic acid (100 μM), during protein expression did not consistently increase activity (data not shown). The absence of an increase in activity upon addition of heme and 5-aminolevulinic acid during expression may be related to hydrogen peroxide not being a substrate, as covalent binding of heme has been shown to

occur as a result of auto-oxidation of the enzyme with hydrogen peroxide. The reducing conditions of the *E. coli* expression system and the likely catalase activity (30) in whole cells could also make hydrogen peroxide limiting. A similar condition was reported (37) for LPO derived from a baculovirus expression system where covalent heme binding was increased by the addition of hydrogen peroxide. Hydrogen peroxide was also required for covalent binding of the heme to LspPOX, a bacterial peroxidase of this family (18).

Studies on the native protein suggested that PQQ may play a role in Mn oxidation (6). The addition of 10 μM PQQ to cell extract assays increased Mn oxidation by 50% with both the full-length MopA and the AHP domain (Table 1). This increase in activity with the heterologously expressed protein is consistent with, but much less than, the 8-fold increase in activity seen with the natively expressed protein in cell extracts (6). Although there is an increase in Mn-oxidizing activity resulting from PQQ addition with both the native and heterologously expressed proteins, the role of PQQ still needs to be elucidated. In contrast to the native system, the addition of 1 mM NAD^+ or NADH to the cell extract assay did not affect activity (data not shown).

Calcium is required for Mn oxidation. Although calcium stimulated activity with the natively expressed protein, it was not required for activity (5). In contrast, calcium proved to be essential for activity of the heterologously expressed MopA and the AHP domain (Table 2). The calcium requirement for activity of both MopA and the AHP domain which lacks the calcium binding domain suggests a role for calcium in the catalytic domain. In fact, the structures of MPO (36) and LPO (34) indicate a bound calcium ion is required for orienting the distal histidine that interacts with the heme cofactor (31). Like MopA and the AHP domain, calcium binding is critical for activity of animal heme peroxidases (38). The residues that bind calcium in MPO and LPO are conserved in MopA and the AHP domain (39).

Determination of kinetic parameters in cell extract. We de-

TABLE 2 K_m and V_{max} values of natively expressed MopA, heterologously expressed full-length MopA, and the heterologously expressed AHP domain in cell extract[a]

Protein or domain	K_m ± SD for Mn(II) (μM)	V_{max} ± SD (nM Mn oxide equivalents · minute^{-1} · mg cell extract^{-1})
MopA from native host	204 ± 43	2.5 ± 0.12 (6)
Full-length MopA	545 ± 278	2.6 ± 0.38
AHP domain	3,624 ± 741	792 ± 52

[a] Values are from triplicates.

termined the K_m for Mn(II) and V_{max} kinetic parameters for the heterologously expressed MopA and the AHP domain in cell extract. The K_m for heterologously expressed MopA in cell extract was similar to that of the natively expressed protein (6) in cell extract (Table 1). Although the cell extract environments of the natively expressed protein and the heterologously expressed protein are likely different and therefore interpretation of the kinetic parameters has obvious limitations, it does provide evidence that we are examining the same protein catalyzing Mn-oxidizing activity. The K_m for the AHP domain was significantly higher than that for the full-length MopA (Table 2). Although the calcium binding domain is not required for activity, it appears to significantly increase the affinity for Mn. It is irresistible to conjecture that the multiple calcium binding sites on this domain may bind Mn(II) to be delivered to the active site. The K_m of the full-length MopA is significantly lower than the K_m determined for the heterologously expressed MCOs CotA and CueO (14.85 ± 1.17 mM [15] and 17.33 ± 3.63 mM [17], respectively), suggesting that MopA activity is likely to be more relevant for biogeochemical cycling in most natural systems.

The V_{max} values for MopA in the native host (6) and when expressed in *E. coli* were similar, approximately 2.5 nM Mn oxide equivalents · minute^{-1} · mg^{-1} (Table 2). One of the great advantages of heterologous expression is the increase in expression of a desired protein, but for the full-length MopA, based on qualitative SDS-PAGE analysis, the protein was expressed at similar levels in the native host and recombinantly, which is consistent with the similar V_{max} values. The AHP domain had a >300-fold-higher V_{max} than MopA (Table 2), likely due to the significant increase in expression (Fig. 2).

The animal heme peroxidase-type Mn-oxidizing enzymes likely play an important role in Mn cycling. This class of Mn-oxidizing enzymes was recently identified (5), and there are many questions regarding how they may function. The experiments described here provide important clues on the enzyme mechanism, which will assist future studies to purify and characterize this intriguing enzyme.

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