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Biological Functions Controlled by Manganese Redox Changes in Mononuclear Mn-Dependent Enzymes

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Abstract

Remarkably few enzymes are known to employ a mononuclear Mn(II) ion that undergoes changes in redox state during catalysis. Many questions remain to be answered about the role of substrate binding and/or protein environment in modulating the redox properties of enzyme-bound Mn(II), the nature of the dioxygen species involved in the catalytic mechanism, and how these enzymes acquire Mn(II) given that many other metal ions in the cell form more stable protein complexes. Here we summarize current knowledge concerning the structure and mechanism of five mononuclear manganese-dependent enzymes: superoxide dismutase, oxalate oxidase, oxalate decarboxylase, homoprotocatechuate 3,4-dioxygenase, and lipoxygenase. Spectroscopic measurements and/or computational studies suggest that Mn(III)/Mn(II) are the catalytically active oxidation states of the metal, and the importance of “second-shell” hydrogen bonding interactions with metal ligands has been demonstrated for a number of examples. The ability of these enzymes to modulate the redox properties of the Mn(III)/Mn(II) couple, thereby allowing them to generate substrate-based radicals, appears essential for accessing diverse chemistries of fundamental importance to organisms in all branches of life.

Introduction

Manganese exhibits a rich and varied chemistry among the first-row transition metals, and has been recruited by biology to perform a remarkable range of catalytic functions [1-4], especially when Mn clusters are present in the active site. For example, the unique redox properties of Mn are essential to the generation of dioxygen from water in the oxygen-evolving complex [2, 5], and in the production of the deoxyribonucleotide building blocks needed for DNA synthesis [6]. In addition, mononuclear Mn(II) ions can be used as Lewis acid catalysts by numerous enzymes of metabolic importance, including glutamine synthetase [7]. It is believed that Mn(II) was present at 50 mM concentration on the “early” Earth, perhaps giving rise to the myriad biological roles played by this transition metal. On the other hand, there appear to be only five enzymes possessing a non-heme, mononuclear Mn(II) center, which changes oxidation state during the catalytic cycle, that have been mechanistically and structurally characterized: Mn-dependent superoxide dismutase (MnSOD) [8], oxalate oxidase (OxOx) [9], oxalate decarboxylase (OxDC) [10], Mn-dependent homoprotocatechuate 2,3-dioxygenase (MndD) [11] and Mn-dependent lipoxygenase (MnLOX) [12]. Even so, direct evidence for the involvement of Mn(III) in the catalytic cycle has only been obtained for two of these enzymes, and whether the dioxygenase needs to employ higher metal oxidation states during catalysis has been the subject of debate.

Bioinorganic chemistry of manganese

Manganese can exist in a wide variety of oxidation states, ranging from Mn(II) up to Mn(VII). Of these, only Mn(II), Mn(III) and Mn(IV) need to be considered in the aqueous environment of a cell [2]. Although being thermodynamically stable due to a d^5 electronic configuration, Mn(II) exhibits weaker affinity for oxygen and nitrogen ligands than Fe(II) and Zn(II) in the Irving-Williams series ($\text{Mn}^{2+} < \text{Fe}^{2+} < \text{Co}^{2+} < \text{Ni}^{2+} < \text{Cu}^{2+} > \text{Zn}^{2+}$) [13]. Mn(II) also exhibits fast ligand exchange kinetics, which means that it can often be replaced easily by other metal ions, such as Mg(II). The molecular mechanisms by which cells ensure that Mn is correctly bound by Mn-dependent enzymes in the presence of other metal ions, such as Zn(II), remain, however, to be clearly elucidated. In eukaryotes, it is possible that metal selection takes place in specific organelles, such as the Golgi, where Mn(II) can be present at

substantially higher concentrations as a result of active transport in eukaryote cells [14]. Experimental studies also suggest that the location of protein folding is critical in the selection of Mn(II) over Cu(II) incorporation in cyanobacteria [15]. The existence of metallochaperones that mediate metal insertion during the folding of Mn-dependent enzymes has not yet been demonstrated.

Mn(III) is a powerful oxidizing agent given that an electron can be readily placed within an empty *d* orbital, as evidenced by a +1.51 V reduction potential (E°) for hexa-aquo Mn(III) *versus* the normal hydrogen electrode (NHE) [16]. In addition, hydrated Mn(II) ions are stable in water under aerobic conditions at neutral pH given the mid-point potential associated with converting oxygen into superoxide anion (Table 1). It is, however, considerably easier to oxidize Mn(II) to Mn(III) in basic solutions because a metal-bound water molecule can lose a proton (pK_a 10.6) to give a hydroxide ligand, which stabilizes the higher charge on the Mn(III) ion. In turn, the increased charge of Mn(III) lowers the pK_a of a second water ligand with the result that the Mn(III) species is further oxidized to Mn(IV), a species that then precipitates as solid MnO₂. Hence, if enzymes are to use manganese in the +3 and/or +4 oxidation states, the redox properties of the metal ion must be controlled carefully by the protein environment if unwanted side reactions are to be prevented. One might also expect that such enzymes exhibit optimum catalytic activity in acidic solutions. A change in ionic radius also takes place on oxidation of Mn(II) (0.97 Å) to Mn(III) (0.785 Å) [17], which results in tighter binding of the metal ion to its ligands and altered coordination preferences; these could also be exploited by proteins to modulate the redox properties of the metal. Of course, accessing higher oxidation states of protein-bound Mn(II) requires an oxidizing agent, and examination of standard reduction potentials (Table 1) suggests that direct conversion of Mn(II) to Mn(III) by reaction with oxygen is precluded. This view is supported by the fact that Mn(II) in many enzymes is completely stable under aerobic conditions. Therefore, metal coordination by substrate is almost certainly required if oxygen, rather than superoxide, is to oxidize Mn(II) directly.

In this review, we will briefly outline what is known about metal coordination, catalytic mechanism and functionally important residues of Mn-dependent superoxide dismutase, oxalate oxidase, oxalate decarboxylase, Mn-dependent homoprotocatechuate 2,3-dioxygenase and Mn-dependent

lipoyxygenase. Our goal is to examine what is known about how metal ligands, both from the protein and substrate, together with “second-shell” residues elsewhere in the active site function to control the oxidation state of the manganese ion. In addition, we will present current thinking about how Mn(III) generates substrate-based radicals to catalyze a number of very different biochemical transformations.

Mn-dependent superoxide dismutase

As part of the cellular response to oxidative stress [18], Mn-dependent superoxide dismutase (MnSOD) catalyzes the disproportionation of two superoxide radical anions to dioxygen and hydrogen peroxide (Figure 1) [8]. The enzyme is a homotetramer in humans and other eukaryotes, but usually exists as a homodimer in prokaryotes [19]. In the resting state of MnSOD, the metal is coordinated in a trigonal bipyramidal geometry by a water molecule and the side chains of three histidines and an aspartate residue (Figure 1). This coordination geometry favors Mn(III), and this form of the metal is present in the “as-isolated” enzyme and can only be removed by partial denaturation of the protein. Presumably Mn(II) is incorporated into the enzyme when it is first produced in the cell, but the nature of the molecular species that oxidizes the metal ion to give Mn(III)-containing SOD remains to be elucidated [20]. A “conformational gating model” explains how changes in pH permit apo-MnSOD to bind Mn(II), which is then oxidized by superoxide in the environment to form Mn(III) [21]. Defining the resting state of the enzyme to be the Mn(III)-containing form, electron transfer to the metal from superoxide radical anion in the initial half-reaction yields dioxygen and enzyme-bound Mn(II). This, in turn, is oxidized back to Mn(III) by a second molecule of superoxide in a proton-coupled electron transfer reaction giving a hydroperoxyl anion. Subsequent protonation then forms hydrogen peroxide (Figure 1) [8]. Heroic measurements have shown that the mid-point potentials (E_m) of the metal in human and *Escherichia coli* MnSOD are +0.39 V and +0.29 V, respectively [22]. These values compare well with the requirement that the E° of the metal must be near to +0.36 V, which is approximately mid-way between the standard reduction potentials for the two half reactions involving superoxide [23] (Table 1). Current proposals suggest that the protein environment alters the pK_a of Mn-bound water to achieve this significant change in metal redox properties [24]. Thus, stabilizing the hydroxide form of the ligand will favor Mn(III) thereby lowering E° , this mechanism being most effective when electron and proton

transfer are coupled [25]. Calculations support such a proposal [26], and it appears stabilization of a hydroxide ligand is accomplished through a “second-shell” hydrogen bonding to the Gln-146 side chain (*Escherichia coli* numbering) [27]. In turn, this residue is part of a hydrogen bonding network with other residues in the protein. Studies of site-specific variants in which this conserved glutamine residue is replaced by other amino acids not only support this hypothesis but also show the importance of the amide side chain for metal ion binding [28].

Oxalate oxidase

Oxalate oxidase (OxOx), an enzyme that appears to be essential for seed germination [29], oxidizes oxalate into CO₂ with the concomitant reduction of dioxygen to hydrogen peroxide (Figure 2) [9, 30]. It is thought to play a part in protecting plants from fungal infections in addition to its (unknown) role in germination [31]. Structural studies on barley (*Hordeum vulgare*) OxOx revealed the active form of the enzyme to be a hexamer in which each monomer has a canonical “cupin” fold (Figure 2). The manganese-binding site, which is occupied by Mn(II) in the isolated enzyme, is composed of the side chains of three histidines and a glutamate residue, and two water molecules in an octahedral arrangement [32]. The X-ray crystal structure of the complex between glycolate (a structural analogue of oxalate) and OxOx [33] suggests that oxalate monoanion binds the metal center in a monodentate fashion. This leaves one coordination site to which the other substrate, dioxygen, might bind in the initial Michaelis complex (Figure 2). Noting that the activity of the Mn(II)-containing form of the enzyme exhibited relatively low activity and unusual, non-stoichiometric “burst” kinetics, Whittaker and co-workers generated Mn(III) and Mn(IV)-containing recombinant OxOx variants using *in vitro* chemical oxidation methods. On the basis of kinetic measurements and spectroscopic characterization of these variants using UV-visible and EPR spectroscopy, it was concluded that Mn(III) was the biologically relevant form of the metal ion that mediates the two-electron oxidation of oxalate to CO₂ [34]. The nature of the *in vivo* oxidant needed to convert Mn(II) in OxOx to Mn(III), the catalytically active species, was not determined, however, although a weak absorption at 450 nm, which could represent the presence of Mn(III) [35], is observed in the purified enzyme only under aerobic conditions. Based on the free energy change for oxidizing oxalate into CO₂ and a formyl radical anion, the redox potential

of the Mn(III)/Mn(II) couple in OxOx is estimated to be in the range of +0.4 V to +1.0 V [34], again showing that the importance of the protein environment in modulating the redox potential of the Mn(III)/Mn(II) couple. We note that Mn(III) will also be stabilized by binding the substrate monoanion. Several catalytic mechanisms for OxOx have been proposed [34,36], but DFT calculations support the view that decarboxylation takes place from a Mn(II)-bound oxalate radical anion to give CO₂ and a formyl radical anion in the initial step (Figure 2) [37]. Coupling of a hydroperoxyl radical with the latter species then yields a peroxyacid intermediate that can spontaneously decompose to CO₂ and hydrogen peroxide. These calculations also showed that dioxygen can only oxidize oxalate-bound Mn(II) to Mn(III) if the former adopts a lower ($S = 3/2$) spin state than the high-spin state ($S = 5/2$) that is present in the purified enzyme. Mutagenesis experiments support the view that Asn-75 and Asn-85 (*Hordeum vulgare* numbering) play a role in orienting the substrate for reaction [34] but further work is needed to identify “second shell” residues that may modulate the redox properties of the manganese center in OxOx. In addition to the OxOx that is present in plants, for which the monomer contains a single cupin domain (Figure 2), an ortholog of this enzyme has also been isolated from the white-rot fungus *Ceriporiopsis subvermispora*, which is composed of two fused cupin domains [38]. Each of these domains contains conserved metal ligand residues for Mn(II) binding site, and it has been shown that Mn(II) is required for activity [39]. Technical difficulties in obtaining sufficient amounts of fully loaded, Mn-containing enzyme by heterologous expression in yeast have precluded direct observation of any Mn(III) during catalytic turnover using spectroscopic methods. It is believed, however, that this ortholog employs the same mechanism as that proposed for *Hordeum vulgare* OxOx.

Oxalate decarboxylase

Oxalate decarboxylase (OxDC) [10,40], which is present in many wood-decaying fungi [41] and bacteria such as *Bacillus subtilis* [42], mediates oxalate degradation via a disproportionation reaction to give CO₂ and formate (Figure 3). Somewhat unexpectedly, OxDC activity requires aerobic conditions even though dioxygen is not consumed in the disproportionation reaction [40]. Although the enzyme has been isolated from fungal sources, almost all of the detailed structural and mechanistic studies have been performed by heterologous expression of *Bacillus subtilis* OxDC in *Escherichia coli* to give

recombinant enzyme containing Mn(II) when purified [10]. Kinetic characterization of a series of transition metal-containing variants of recombinant *Bacillus subtilis* OxDC show that the enzyme has an absolute requirement for manganese if it is to be catalytically active [43]. X-ray crystallography shows *Bacillus subtilis* OxDC to be a hexamer, composed of two trimeric units built from interlocked monomers (Figure 3) [44,45]. As observed for *Ceriporiopsis subvermispora* OxOx [39], each OxDC monomer is built from two cupin domains that both contain a Mn(II)-binding site comprised of three histidines and a glutamate residue (Figure 3). Two water molecules occupy the remaining sites in the octahedrally coordinated Mn(II) ion. The results of mutagenesis experiments suggest that catalytic activity is only associated with the N-terminal Mn(II)-binding site [46, 47], and additional support for this hypothesis has been provided by the X-ray crystal structure of oxalate bound to a cobalt-substituted OxDC variant [48]. The dependence of decarboxylase activity on Mn-occupancy [43], however, suggests that Mn-binding in the C-terminal binding site is required for optimal activity, although the exact role played by metal binding within the C-terminal domain remains to be determined. On this point, although the metal binding sites of N- and C-terminal domain are almost identical, differences in the “second shell” residues, such as Trp132, which has hydrogen bonding interaction with the N-terminal metal ligand Glu101, may modulate the electronic structure of the two Mn(II) ions on the basis of EPR measurements. [49] Heavy atom isotope effect measurements suggest that C-C bond breaking proceeds in a heterolytic fashion [50], presumably from a Mn-bound oxalate radical anion [51]. The latter is almost certainly generated after substrate binding by electron transfer to Mn(III) (Figure 3), and direct observation of Mn(III) during catalytic turnover by high-field, parallel mode EPR measurements support this mechanistic proposal [52]. As in the case of OxOx, however, the molecular species that actually oxidizes the Mn(II) that is present in the purified, recombinant enzyme remains unclear. Given that dioxygen is required for catalytic activity, current mechanistic hypotheses postulate metal oxidation by this species to give an initial superoxide radical and Mn(III) complex [50-53]. Given that the purified Mn(II)-containing enzyme is not oxidized in the absence of oxalate, such a model suggests that binding the negatively charged oxalate monoanion, which is the true substrate [50], modulates the redox properties of the Mn(III)/Mn(II) couple to facilitate dioxygen-mediated oxidation to yield the catalytically active form of the enzyme. This model therefore provides a role for dioxygen in catalysis even though it

is not required for the disproportionation reaction. It is also possible that interactions between metal ligands and “second shell” residues might modulate the redox properties of the Mn(II) ions and associated catalytic activity [49]. For example, the side chain of a conserved tryptophan residue hydrogen bonds to Glu-101, which coordinates the metal in the N-terminal domain of the enzyme. In the C-terminal site, however, a similar hydrogen bond is formed instead between Gln-282 and Glu-280. Such interactions may overcome the difficulty of oxidizing the Mn(II) to Mn(III) when combined with binding the negatively charged substrate to the metal center. The nature of the oxidizing species, however, remains controversial and it is quite possible that something other than dioxygen, such as superoxide, mediates the formation of Mn(III) during catalytic turnover [53, 54]. Mutagenesis studies have also shown that “second shell” residues, such as Arg-92, Glu-162 and Thr-165, play important roles in positioning the substrate and controlling the accessibility of the active site so as to protect the radical intermediates formed during catalysis (Figure 3) [46,48].

Mn-dependent homoprotocatechuate 2,3-dioxygenase

Mn-dependent homoprotocatechuate 2,3-dioxygenase (MndD) converts aromatic compounds (xenobiotics) from the environment into intermediates used in primary metabolism [11, 55]. The enzyme is remarkable in that dioxygenases generally employ Fe(II) to form the activated species needed to oxidize substrates, presumably because of the matched redox potentials of Fe(II) and dioxygen [56]. Structural studies on MndD from soil bacteria *Arthrobacter globiformus* have shown that the Mn(II) ion in the resting enzyme is coordinated by the side chains of two histidine residues and a glutamate, which are all conserved (Figure 4) [57]. The remaining three octahedral coordination sites are occupied by water. As observed for Fe-dependent catechol dioxygenases [58], substrate binding displaces two of these Mn-bound waters [59] with the third site being occupied by dioxygen in the active form of the Michaelis complex. Somewhat unusually, substitution of Fe(II) for Mn(II) was reported not to impact dioxygenase activity, leading to the initial conclusion that Mn(II) did not change oxidation state during the catalytic cycle [59]. Subsequent EPR experiments, however, showed that dioxygen binding to the substrate-coordinated metal ion did generate a short-lived Mn(III)/superoxide radical anion complex that underwent reaction with the substrate (Figure 4) [60]. It is believed that electron

transfer to dioxygen from the metal must, therefore, take place faster than from the substrate because the planar catechol needs to undergo a geometric change to the non-planar semiquinone intermediate (Figure 4) [60]. Computational studies support the intermediacy of Mn(III) in the catalytic mechanism [61, 62]. Less is known about how the protein modulates the redox properties of the metal, although, as in the case of OxDC [52], the binding of the substrate anion likely stabilizes Mn(III). Unlike the metal centers in MnSOD, OxOx or OxDC (Figures 1-3) in which three conserved histidines are directly coordinated to the metal, a third histidine (His-200) is positioned approximately 3.9 Å away from the Mn center that is thought to hydrogen bond to superoxide during catalysis [63]. As a result, this “second-shell” residue may facilitate electron transfer between the metal and dioxygen. This proposal has been supported in studies of a Co(II)-substituted variant of the Fe-dependent homoprotocatechuate 2,3-dioxygenase [64], which exhibits higher catalytic activity than the Fe(II)-containing enzyme in dioxygen-saturated buffers although K_M for oxygen is significantly increased. Given that the mid-point potential of Mn(III)/Mn(II) lies between Co(III)/Co(II) and Fe(III)/Fe(II) (Table 1), it is possible that Co(II)-substituted MndD would also exhibit catalytic activity.

Mn-dependent lipoxygenase

Lipoxygenases (LOXs), which have been found bacteria, fungi, plant, and animals, perform a wide range of biological functions by oxidizing polyunsaturated fatty acids into hydroperoxides [65]. The non-heme Fe-dependent LOXs (FeLOXs) present in mammalian cells have been well studied because they synthesize lipid hydroperoxides, which are involved in signaling, cancer development, and inflammation [66]. Plant pathogenic fungi, however, express and secrete a unique, highly glycosylated LOX that is Mn-dependent (MnLOX) [67]. Interestingly, the catalytic mechanism of MnLOX differs from that employed by FeLOX in two ways. First, MnLOX can oxidize bis-allylic methylene carbon atoms at a much faster rate, and hydrogen abstraction/dioxygen insertion take place in a suprafacial, rather than an antarafacial, manner [68, 69]. These mechanistic effects may be a consequence not only of differences in active site structure but also the very different redox properties of Mn(III)/Mn(II) and Fe(III)/Fe(II) couples [69]. A recent X-ray crystal structure of recombinant MnLOX, present in the rice blast fungus *Magnaporthe oryzae*, shows that Mn(II) is coordinated by a water molecule, three histidine

residues, an asparagine side chain, and the carboxylate group of a C-terminal valine residue in a distorted octahedral arrangement (Figure 5) [70]. The use of the C-terminal “backbone” carboxylate is an interesting variation from coordinating the metal with aspartate and glutamate side chains, but this ligand has also been observed in Fe-dependent 8*R*-lipoxygenase [71]. Differences in the interaction of metal ligands with “second-shell” residues are observed in MnLOX and FeLOX, which might account for the observed differences in reactivity. For example, a hydrogen bond between the side chain of Gln281 and that of Asn473, which directly binds the metal, has been proposed to play a role in differentiating the catalytic activity of the Mn(II)- and Fe(II)-containing enzymes [70]. Mutagenesis studies on the MnLOX remain to be reported, however, that validate this structure-based proposal. Current mechanistic proposals invoke dioxygen binding, with a displacement of the Mn-bound water, leading to the formation of Mn(III) and a superoxide radical, which can then abstract hydrogen from the bis-allylic methylene of the lipid. The involvement of a Mn(III)-hydroperoxide in hydrogen atom abstraction, however, has been proposed on the basis of model studies and DFT calculations [72]. There is little direct experimental evidence for the formation of Mn(III) during catalytic turnover, although the addition of substrate results in a decreased Mn(II) signal in the perpendicular mode EPR spectrum of MnLOX due to oxidation of the metal ion [73]. More detailed investigations into the role of manganese in catalysis are eagerly awaited, especially in light of the newly obtained X-ray crystal structure.

Conclusions

Despite the position of manganese in the Irving-Williams series, which complicates the specific incorporation of manganese into proteins, the unique chemistry of this transition metal is still employed to mediate a number of critical biochemical reactions. For the mononuclear enzymes discussed in this review, which likely all employ a Mn(III)/Mn(II) couple in catalysis, the metal ion is coordinated by at least two histidine residues and a carboxylate anion. Differences in coordination number seem to reflect the nature of the substrate that interacts with the metal so that there is always a vacant site into which an oxidant, such as dioxygen or superoxide, can bind. Although the standard reduction potential of the Mn(III)/Mn(II) couple favor the higher oxidation state, the mid-point potential is likely “tuned” by a

complicated combination of active site electrostatics, substrate binding and hydrogen bonding interactions between “second shell” residues in the protein and the metal ligands. Our poor understanding of such effects in Mn-dependent enzymes such as OxOx and OxDC might also underlie, at least in part, the limited success reported for efforts to obtain inorganic Mn complexes capable of catalyzing the breakdown of oxalate [74-76]. It seems clear, however, that Mn(III) formation as a prelude to the generation of substrate-based radicals allows Nature to access diverse chemistries of fundamental biological importance.

Summary

- Mn(III) is a powerful oxidizing agent which can be used for redox catalysis in enzymes by generating reactive substrate-based radicals.
- Mn(III)/Mn(II) have been proposed to be the catalytically active oxidation states of the metal in MnSOD, OxOx, OxDC, MndD, and MnLOX on the basis of spectroscopic measurements and/or computational studies.
- At least two histidine and one carboxylate ligand coordinates the manganese ion, which cannot be exchanged with other ions without denaturing the protein. This raises questions about the molecular mechanisms controlling metal selectivity in the cell.
- The importance of second-shell residues in catalysis and tuning the redox potential of the Mn(III)/Mn(II) couple has been demonstrated for two of these enzymes.

Abbreviations EPR, electron paramagnetic resonance; FeLOX, Fe-dependent lipoxygenase; LOXs, lipoxygenases; MndD, Mn-dependent homoprotocatechuate 2,3-dioxygenase; MnLOX, Mn-dependent lipoxygenase; MnSOD, Mn-dependent superoxide dismutase; NHE, normal hydrogen electrode; OxDC, oxalate decarboxylase; OxOx, oxalate oxidase

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Competing Interests

The Authors declare that there are no competing interests with the manuscript.

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Figure 1. Manganese-dependent superoxide dismutase (MnSOD). (Top) Cartoon representations of the biological assembly of the human MnSOD tetramer (left), the human MnSOD monomer (middle), and the Mn center and selected second-shell residues in the active site of human MnSOD (PDB ID: 2QKC [77]). The Mn ion and water oxygen are rendered as purple and red spheres, respectively. All structural representations in Figures 1-5 were created using PyMOL [78]. (Bottom) Proposed mechanism for MnSOD (redrawn based on material in ref. 79).

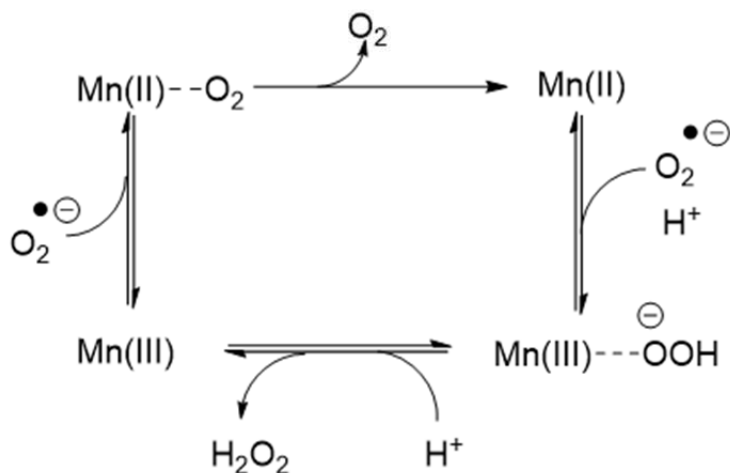


Figure 2. Oxalate oxidase (OxOx). (Top) Cartoon representations of the biological assembly of the *Hordeum vulgare* OxOx hexamer (left), the *Hordeum vulgare* OxOx monomer (middle), and the Mn center and selected second-shell residues in the active site of *Hordeum vulgare* OxOx (PDB ID: 2ET1 [34]). The Mn ion and water oxygen are rendered as purple and red spheres, respectively, and bound glycolate (a substrate analog) has carbon atoms shown in cyan. (Bottom) Proposed mechanism for OxOx (redrawn based on material in ref. 35).

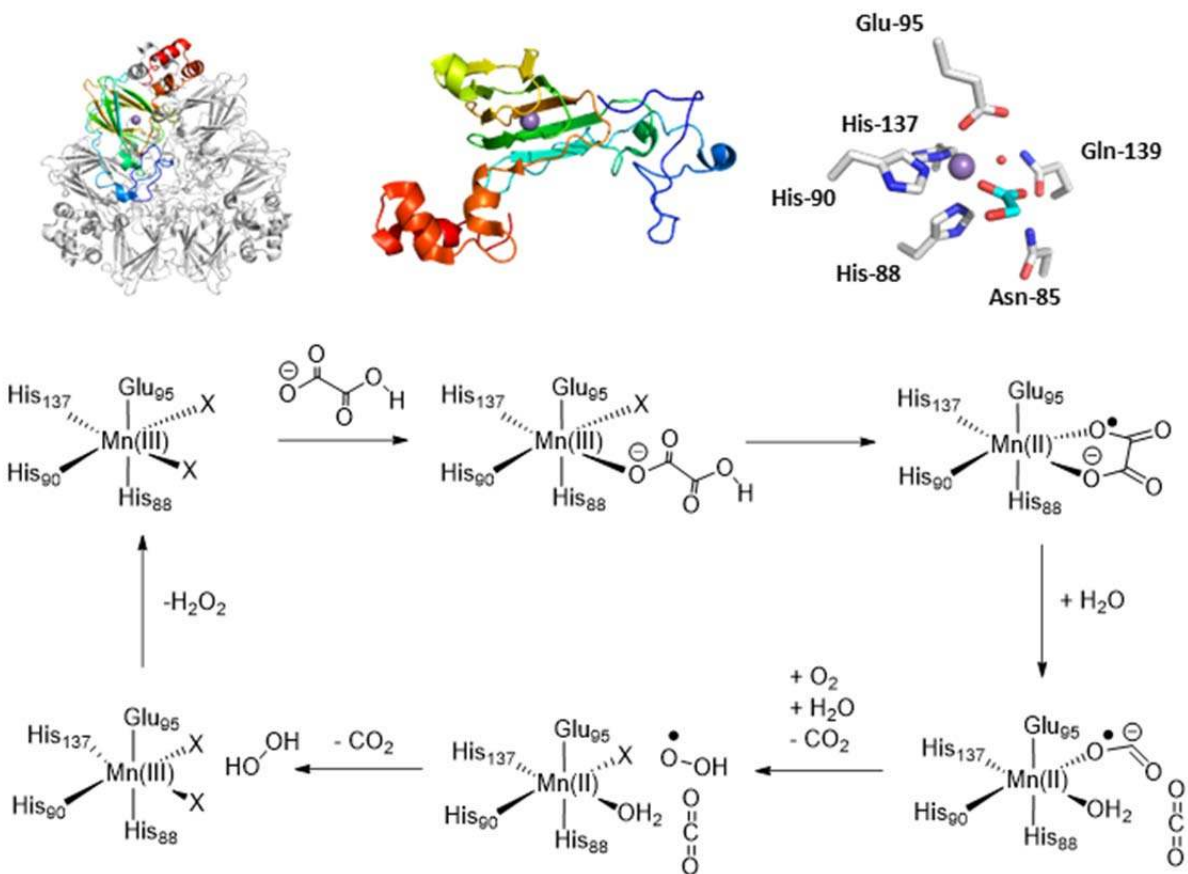


Figure 3. Oxalate decarboxylase (OxDC). (Top) Cartoon representations of the biological assembly of the *Bacillus subtilis* OxDC hexamer (left), the *Bacillus subtilis* OxDC monomer (middle), and the Mn center and selected second-shell residues in the active site of *Bacillus subtilis* OxDC (PDB ID: 1UW8 [45]). The Mn ion and water oxygen are rendered as purple and red spheres, respectively. Mn-bound oxalate, with carbon atoms shown in cyan, is modeled into this structure based on its location in the X-ray crystal structure of a Co(II)-substituted OxDC variant (PDB ID: 5HI0 [48]). (Bottom) Proposed mechanism for OxDC (redrawn based on material in ref. 52).

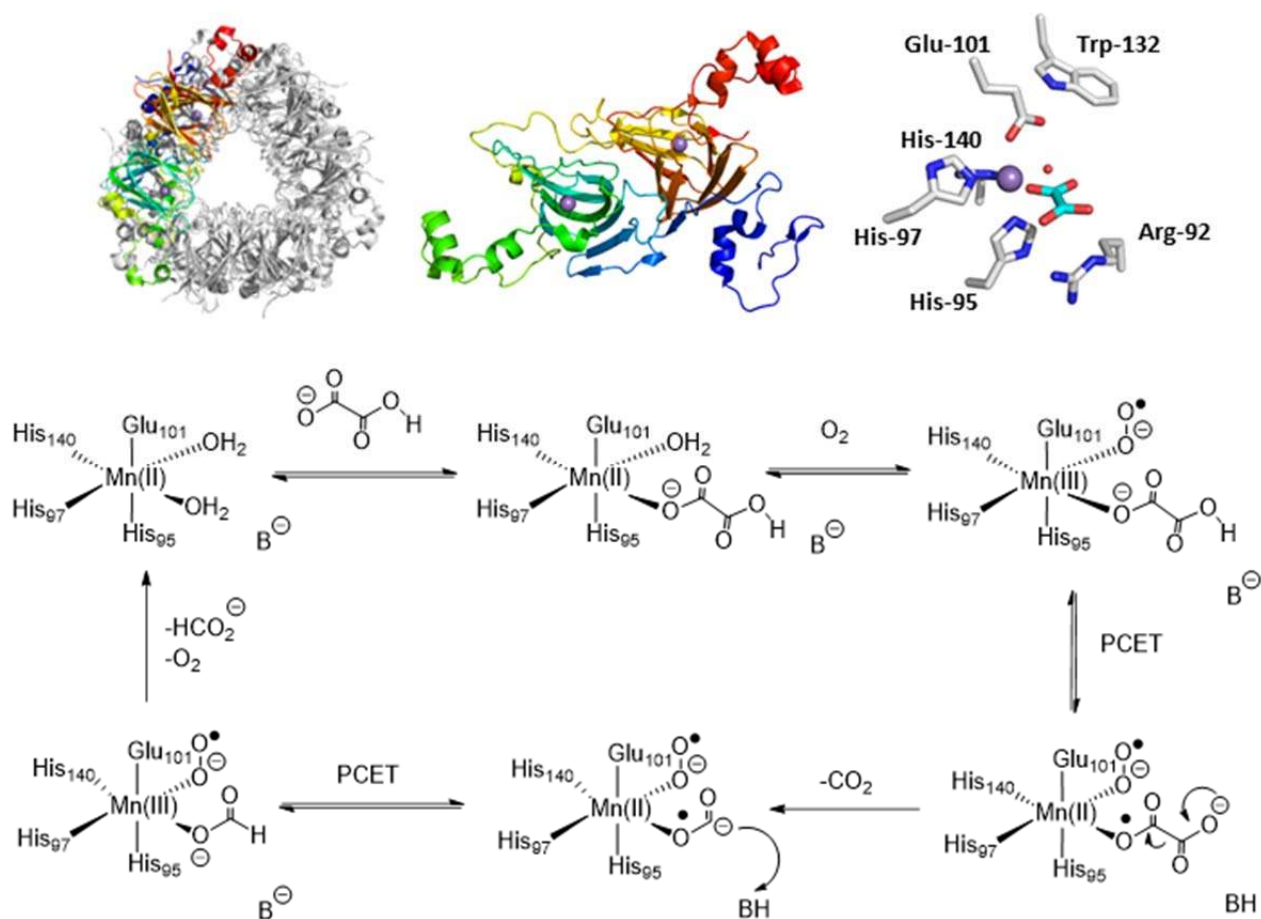


Figure 4. Manganese-dependent extradiol dioxygenase (MndD). (Top) Cartoon representations of the biological assembly of the *Arthrobacter globiformis* MndD tetramer (left), the *Arthrobacter globiformis* MndD monomer (middle), and the Mn center and selected second-shell residues in the active site of *Arthrobacter globiformis* MndD (PDB ID: 1F1V [57]). The Mn ion and water oxygen are rendered as purple and red spheres, respectively, and Mn-bound 3,4-dihydroxyphenylacetic acid has carbon atoms shown in cyan. (Bottom) Proposed mechanism for MndD (redrawn based on material in refs. 59, 61 and 64).

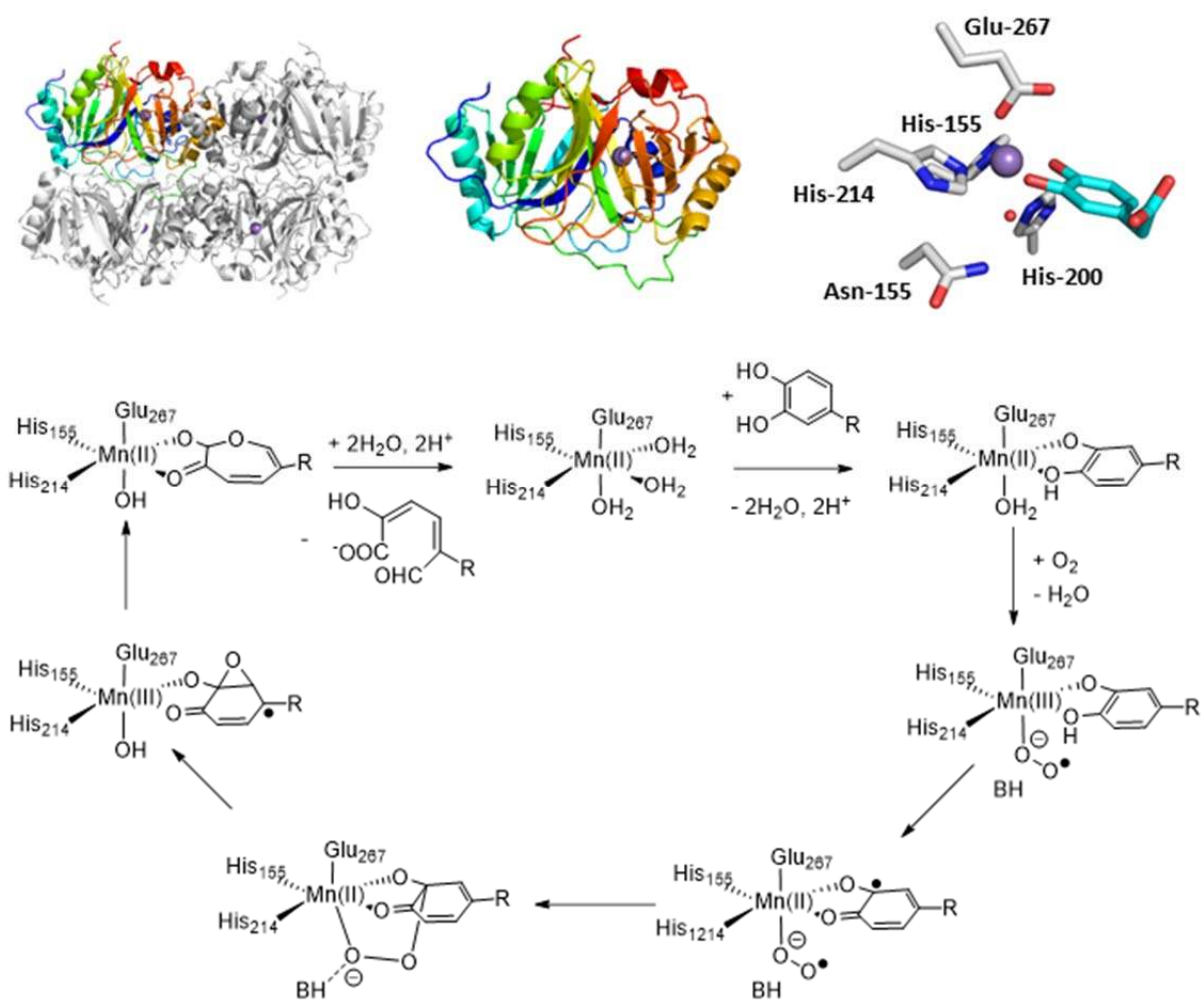


Figure 5. Manganese-dependent lipoxygenase (MnLOX). (Top) Cartoon representations of *Magnaporthe oryzae* MnLOX (left), and the Mn center and selected second-shell residues in the active site of *Magnaporthe oryzae* MnLOX (PDB ID: 5FNO [70]). The Mn ion and water oxygen are rendered as purple and red spheres, respectively, respectively. (Bottom) Proposed mechanism for MnLOX (redrawn based on material in ref. 73).

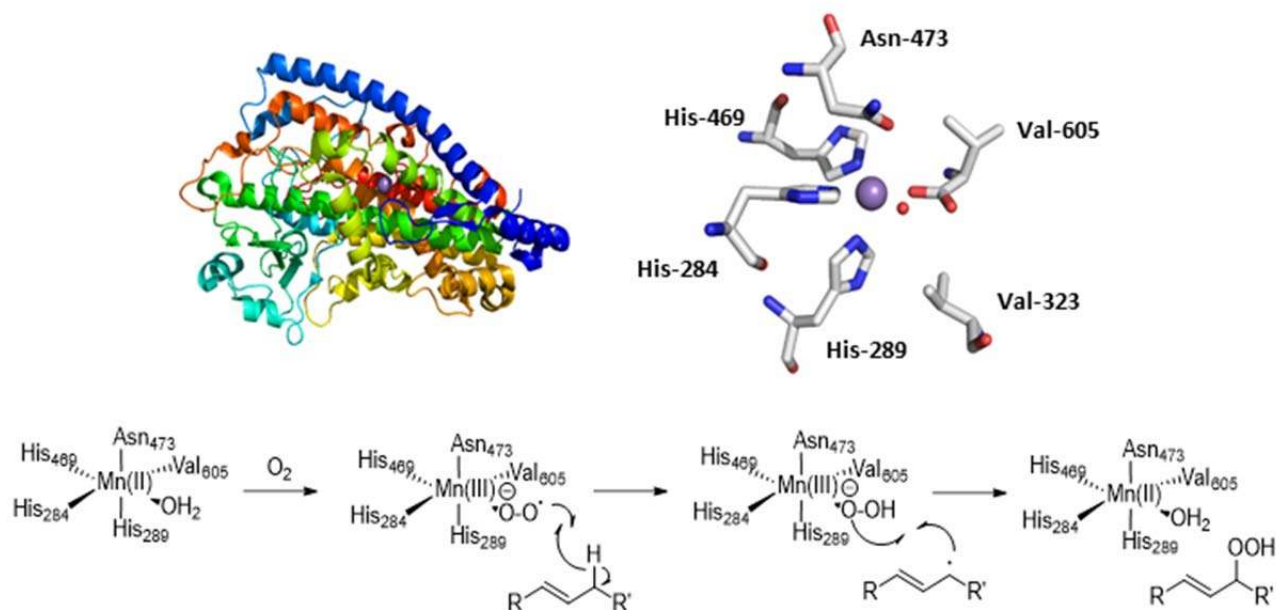


Table 1. Standard reduction potentials at 25 °C.

Half-reaction	E° or E° (V)	pH	Ref.
$\text{Mn}(\text{H}_2\text{O})_6^{3+} + \text{e}^- \rightleftharpoons \text{Mn}(\text{H}_2\text{O})_6^{2+}$	+1.56	0	[80]
$\text{Mn}(\text{OH})_3 + \text{e}^- \rightleftharpoons \text{Mn}(\text{OH})_2 + \text{HO}^-$	+0.05	14	[80]
$\text{Co}(\text{H}_2\text{O})_6^{3+} + \text{e}^- \rightleftharpoons \text{Co}(\text{H}_2\text{O})_6^{2+}$	+1.92	0	[80]
$\text{Fe}(\text{H}_2\text{O})_6^{3+} + \text{e}^- \rightleftharpoons \text{Fe}(\text{H}_2\text{O})_6^{2+}$	+0.77	0	[80]
$\text{O}_{2(\text{g})} + \text{e}^- \rightleftharpoons \text{O}_2^-$	-0.33	7	[81]
$\text{O}_{2(\text{aq})} + \text{e}^- \rightleftharpoons \text{O}_2^-$	-0.16	7	[82]
$\text{O}_{2(\text{aq})} + \text{H}^+ + \text{e}^- \rightleftharpoons \text{HO}_2^{\cdot}$	-0.29	7	[83]
$\text{O}_{2(\text{aq})} + 2\text{H}^+ + 2\text{e}^- \rightleftharpoons \text{H}_2\text{O}_2$	+0.36	7	[83]
$\text{O}_{2(\text{g})} + 2\text{H}_2\text{O} + 4\text{e}^- \rightleftharpoons 4\text{HO}^-$	+0.40	14	[80]
$\text{O}_{2(\text{aq})} + 4\text{H}^+ + 4\text{e}^- \rightleftharpoons 2\text{H}_2\text{O}$	+0.82	7	[82]
$\text{O}_{2(\text{aq})} + 4\text{H}^+ + 4\text{e}^- \rightleftharpoons 2\text{H}_2\text{O}$	+1.27	0	[80]
$\text{O}_{2(\text{g})} + 4\text{H}^+ + 4\text{e}^- \rightleftharpoons 2\text{H}_2\text{O}$	+1.23	0	[80]
$\text{HO}_2^{\cdot}(\text{aq}) + \text{H}^+ + \text{e}^- \rightleftharpoons \text{H}_2\text{O}_2$	+1.02	7	[83]
$\text{O}_2^-(\text{aq}) + 2\text{H}^+ + \text{e}^- \rightleftharpoons \text{H}_2\text{O}_2$	+0.89	7	[84]

