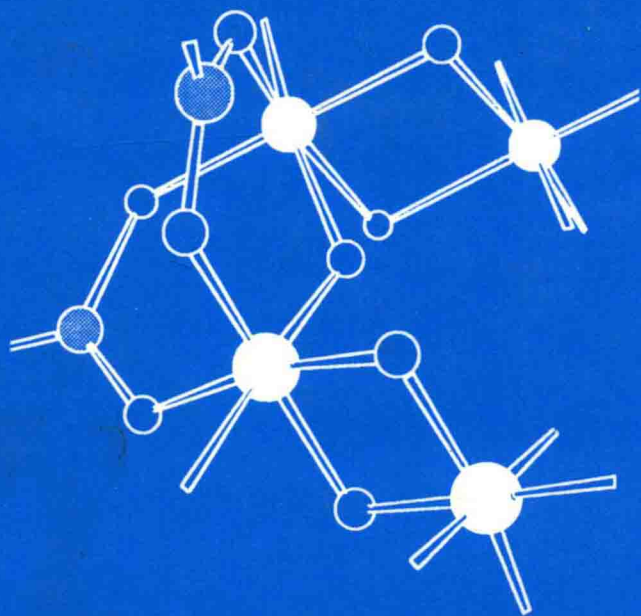


Manganese Redox Enzymes

Edited by
Vincent L. Pecoraro



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MANGANESE REDOX ENZYMES

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MANGANESE REDOX ENZYMES

Preface

In 1958, G. C. Cotzias noted “Mangania is the Greek word for magic, or in modern parlance, voodooism. If this is the root of the metal’s name, it reflects some reality in the biology of manganese, which is rich in phenomena and lacking in adequate guiding principles.” Although thirty years have passed, fundamental advances in our understanding of these principles and their application to the biochemistry of manganese has just begun. After discovering my interest in manganese chemistry, a visiting scholar noted five years ago that “manganese chemistry was easily described: the chemistry of manganese(II) was boring, the chemistry of manganese(III) was tricky and that manganese(IV) existed as the dioxide.” After only two years, the intensity of research into high oxidation state, multinuclear manganese complexes destroyed this simple notion. Today research groups around the world are attempting to model the structure of the active centers and reactivity of the different redox manganoenzymes that have recently been discovered.

Our knowledge of the biochemistry of manganese has also made remarkable progress in the last ten years. It is now established that manganese is essential for the water splitting reaction in higher plant and algal photosynthesis. Non-heme manganese enzymes that detoxify hydrogen peroxide and superoxide have been isolated. The systems can be quite complex, for example, requiring additional inorganic cofactors such as chloride and calcium or shuffling manganese through multiple oxidation states during catalysis. Further definition of the biological properties of manganese has hinged upon the increasingly more highly resolved and purified preparations of these enzymes.

The biophysics of manganese systems are fascinating. Unique epr spectral features that are the hallmark of multinuclear manganese centers have been observed both in the photosynthetic oxygen evolving complex and the manganese catalase. X-ray absorption spectroscopy has been applied to the problem, resulting in detailed structural data for some of these elusive systems. The application of X-ray crystallography promises to define structure, if not help evaluate the mechanism, of these mysterious enzymes.

This book attempts to present the most recent advances in the field of redox manganoenzymes with a heavy emphasis on the oxygen evolving complex. To successfully accomplish this goal requires a detailed coverage of topics. The reader is first introduced (chapter 1) to the broad range of manganese enzymes including those that transform superoxide into hydrogen peroxide and water (superoxide dismutase) and hydrogen peroxide into water and dioxygen (catalase) with chapter 2 presenting the latest results on the enzymology of the *L. plantarum* catalase. From this point the

biochemical, biophysical and synthetic approached to define the chemistry of the photosynthetic oxygen evolving complex are explored. Chapters 3 and 4 examine the biochemical aspects of the system using alternate substrates as probes for mechanism and delineating the interaction between other incofactors (halides and calcium) with the manganese ions. Chapters 5–9 provide a broad range of biophysical techniques that have been employed to ferret out the structure and mechanism of the OEC. These chapters describe optical, epr, EXAFS and NMR spectroscopic experiments that have been crucial in defining the structure and kinetic behavior of the photosynthetic system. The remaining chapters (10–12) provide advances in the synthetic modelling of the OEC and manganese catalase. Furthermore, chapter 11 illustrates how manganese can be used to understand the chemistry of nonmanganese containing systems. Because the study of manganese enzymes is a vibrant area, not all workers in the field could be or were able to contribute to this volume; however, a broad representation of the techniques and viewpoints of this area are presented. When completed, the reader will see that manganese lives up to its name as a magician and is some times tricky, but never boring.

January 1992

V. L. P.

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Introduction to Manganese Enzymes

Erlund J. Larson and Vincent L. Pecoraro

A wide variety of biochemical transformations utilize manganese as a cofactor. In particular, the past 10 years have been witness to the discovery of numerous enzymes whose apparent functions delineate the importance of this element in the metabolism of dioxygen and its reduced forms. While many proteins requiring divalent manganese exhibit low metal ion specificity, redox manganese enzymes exhibit a high specificity for manganese and often make use of the metal in its higher oxidation states. Primary examples of systems of this type are the oxygen-evolving complex in photosystem II, the manganese catalases, and superoxide dismutases.

A great deal of biochemical and biophysical data have now been collected on some of these systems, whereas for others knowledge is still scant. Accompanying this effort has been a surge in the study of the basic coordination chemistry of manganese, especially of its higher oxidation states in environments considered biologically relevant. The complimentary information gained by these inquiries has allowed for significant narrowing of structural possibilities and created sound footing on which reasonable mechanisms may be proposed.

This chapter provides a brief overview of the basic coordination chemistry and spectroscopy of manganese, with emphasis on how these properties may affect its biological activity. A brief description is presented of the known roles of manganese with emphasis on those systems that have a strict requirement for this element as a redox-active constituent. Finally, some recent synthetic models for the oxygen-evolving complex (OEC) and the manganese catalase will be discussed.

Coordination Chemistry of Manganese

It is important to recognize the general coordination properties of manganese before attempting to interpret biophysical data pertaining to the active sites of manganoenzymes. With detailed knowledge of this coordination behavior, theories on the relationship between structure and function in more complex systems can be set forth and tested.

Manganese is an element of relatively high abundance in the earth's crust (0.085%)

Table 1.1. Relevant Physical Properties of Manganese Ions¹⁰⁷

	Electronic Configuration	Spin Only Magnetic Moment	Coordination Preferences (#)	Lewis Acidity	Typical Exchange Rate
Mn (II)	d^5	5.9	6	Moderate	10^7 s^{-1}
Mn (III)	d^4	4.9	5, 6 ^a	Strong	10^5 s^{-1}
Mn (IV)	d^3	3.9	6	Very strong	10^{-2} s^{-1}

^aJahn–Teller distorted octahedron, usually a tetragonal distortion (D_{4h}).

where it is found mainly in the form of oxides, hydrous oxides, and carbonates.¹ The element can adopt a wide variety of formal oxidation states but is most often found in the Mn^{2+} to Mn^{7+} levels. Due at least in part to the highly oxidizing nature of 5+, 6+, and 7+ with respect to protein based ligands, the 2+, 3+, and 4+ ions are most often utilized by biological systems. An outline of the major properties of these ions can be found in Table 1.1.

Manganese acts as a strong Lewis acid in its 2+, 3+, and 4+ oxidation states. It then prefers hard Lewis bases as donors. In a biological milieu, this criterion may be satisfied by hydroxide or oxide ligands as well as alkoxides, phenoxides, and carboxylates donated from protein side chains. Nitrogen donors such as imidazole and sulfur donors are softer Lewis bases but can also function as ligands to manganese.

The donor capacity of the ligands to manganese directly affects the electropositivity of the metal center. Therefore, the redox potential and the subsequent ability of the ion to act as an oxidant or reductant are strongly dependent on the nature of the ligands. In general, Mn(IV) is a strong oxidant and a poor reductant. In part this accounts for the relative rarity of known stable Mn(IV) species. However, the use of strongly donating hard Lewis bases such as oxides, alkoxides, and phenoxides can greatly moderate the potential of Mn(IV).² The Mn(III) ion in most environments is of intermediate potential and can act both as an oxidant or reductant. These characteristics are largely a result of the d^4 electronic configuration. In an octahedral environment, the high spin d^4 configuration places one electron in the antibonding e_g set of orbitals. Loss of this electron generates the d^3 configuration having maximum ligand field stabilization energy (LFSE). Also, addition of an electron creates the stable half filled shell (d^5) configuration, maximizing electron exchange energy.

For Mn(II)(d^5), the loss of this electron exchange energy accounts for its poor reducing ability. In addition, Mn(II) is also a very poor oxidant because the addition of a sixth d -electron causes a loss in exchange energy and requires the expenditure of pairing energy. As was noted for Mn(IV), the precise type of ligands can change the potential of the metal over a wide range within a given formal oxidation state. In this way the metal's environment interacts strongly to either augment or moderate the aforementioned inherent redox tendencies.

Another mode of communication between the metal ion and its environment is the differential preferences between oxidation states for coordination symmetry and number. The charge, size, and number of d electrons on the metal all have direct effects on its preferred coordination number and geometry. Manganese 2+, 3+, and

4+ are found in general to employ five or six ligands in their first coordination sphere [Mn(II) is also found tetrahedral], giving rise to either octahedral or square pyramidal derived geometries. The complexes thus formed are almost invariably high spin (except in cases possessing very strong field ligand sets).¹ In environments having these symmetries, a simple ligand field model demonstrates that Mn(II), Mn(III), and Mn(IV) have very different specific coordination preferences (see Table 1.1).

High spin Mn(II) has five unpaired *d* electrons, one occupying each of the *d* orbitals, giving rise to zero LFSE in all geometries. Since the ion has no electronically imposed geometric preferences, formation of octahedral (and tetrahedral) complexes, which minimize ligand–ligand repulsions, is most often seen. The equilibrium constants for their formation are relatively low since Mn(II) is a larger divalent cation than those of Fe, Co, Ni, or Cu.¹ Its size also causes it to form predominantly six coordinate complexes.

The high spin Mn(III) ion possesses four *d*-electrons, which in strict octahedral symmetry give rise to a net $-6Dq$ LFSE. Increased charge on the manganese ion causes a preference for five or six coordination. However, since the fourth electron is contained in the doubly degenerate e_g set of *d* orbitals (antibonding), one expects a first-order Jahn–Teller distortion away from strict octahedral symmetry.³ Some distortions away from the octahedron are seen which involve intermediate symmetries along a Bailar-type distortion pathway toward a trigonal prism via rotation about a 3-fold axis of the octahedron.⁴ However, most often the distortion is seen to occur through a tetragonal elongation along one of the axes that breaks the degeneracy of the e_g set, minimizing the overall potential energy of the system. An extension of this distortion can be seen in the adoption of five coordinate square pyramidal structures.⁵ Other five coordinate geometries such as the trigonal bipyramid are also seen.⁶ Thus, as a result of the *d*-electron configuration, Mn(III) lacks a strong geometric preference and in contrast to Mn(II), does not adopt pure octahedral geometry.

Removal of the fourth *d*-electron from manganese eliminates the possibility of a Jahn–Teller effect and Mn(IV) enjoys the highest LFSE in strict octahedral symmetry ($-12Dq$). For this reason Mn(IV) shows a much stronger preference for the octahedron than Mn(II) or Mn(III).²

Due to this disparity in the coordination preferences for the different formal oxidation states of manganese, redox processes are often accompanied by significant first coordination sphere rearrangements. This effect manifests itself in the electrochemical studies of small complexes where transformations between the 2+, 3+, and 4+ are often seen to be quasireversible or irreversible.⁷ For manganese enzymes being studied, it is of primary importance to understand how the structural environment fostered by the protein accommodates these changes in such a way as to utilize their thermodynamic tendencies, possibly to impart directionality to a catalytic cycle.

Another consequence of the different electronic configurations of the 2+, 3+, and 4+ states is that their ligand exchange rates are markedly different. The rate of exchange is strongly dependent on the magnitude of ligand field stabilization energy lost on formation of the transition state. Since most ligand-exchange processes involving the octahedron are dissociative, the intermediate activated complex is five coordinate.¹ Both Mn(II) and Mn(III) suffer little loss in LFSE on formation of a five

coordinate complex while the Mn(IV) ion is strongly destabilized relative to six coordination. Therefore, considering LFSE only, one would predict much slower exchange kinetics for Mn(IV). Other factors also contribute to the observed kinetics, perhaps the most significant being the energy required to break the electrostatic interaction between a neutral dipole or anion and the metal. This energy requirement generally increases with the formal charge on the metal, further hindering the process for Mn(IV). The observed exchange rates vary over nine orders of magnitude⁸ with a more drastic decrease on reaching the Mn(IV) level, as shown in Table 1.1. Once again this information is critical to postulating likely mechanistic pathways for the enzymatic systems.

Magnetic Properties

As for other proteins having transition metal-containing active sites, the magnetic properties of manganese and their spectroscopic consequences have proved to be powerful handles that have provided direct insight on the active sites of manganoenzymes. Since spin orbit coupling in manganese is small, the magnetic moments of mononuclear manganese ions usually remain virtually unperturbed from their spin only values.

Many manganese enzymes have been shown to contain multinuclear centers in which the ions interact magnetically. The magnetic behavior of these systems is much more complex. In these cases the net moment of the cluster at any temperature is dependent on the extent of interaction between the individual metal centers. The magnitude of this interaction can be determined by fitting the variable temperature magnetic susceptibility to a form of the Van Vleck equation.⁹ The values obtained are usually reported as J or $2J$ in units of cm^{-1} . Negative J values are by convention used to indicate antiferromagnetic coupling (net spin pairing leading to lower net magnetism) and positive values denote ferromagnetic interaction. The type of coupling depends on many factors including the mode of exchange, spin states of the ions, orbital configurations, etc. In general, biologically relevant complexes of manganese exhibit weak to moderate antiferromagnetic coupling^{9,10} with J values ranging from -1 to -400 cm^{-1} , which leaves residual paramagnetism at room temperature even in net integral spin systems. These are small values compared to the typically more covalent copper species which can have $J = -1200 \text{ cm}^{-1}$ making them diamagnetic at room temperature.¹¹

Magnetic exchange between metals is propagated in numerous ways. The most obvious and efficient of these is direct metal-metal bonding. This mode of exchange is *not* generally seen in biologically relevant manganese complexes. Superexchange through bridging ligand orbitals is the most common and effective route for these complexes. Typical one atom bridges include chloride, oxide, alkoxide, or phenoxide while acetates are very common three atom bridges. The magnitude of interaction depends on the degree of orbital overlap between the magnetic orbitals of the metals and the ligand orbitals. It is therefore sensitive to the type of ligand as well as geometry. Small changes in bridging angle can therefore lead to large changes in magnetic interaction. Likewise, the bond length perturbations associated with oxida-

tion state changes can also lead to differences in magnetic exchange. In general, an increase in formal oxidation state leads to shorter bond lengths and a more effective spin exchange pathway. However, this is not always the case since the orientation of the magnetic orbitals on the metal can be changed by oxidation or reduction. If an electron is formally removed from a magnetic orbital involved in the exchange interaction, the superexchange pathway may be drastically altered, offsetting any bond length effects. Thus even the magnetic properties of Mn dimers are very complex as has been discussed by Girerd et al.¹¹ For higher nuclearity clusters, accurate description of the magnetic behavior becomes extremely difficult since multiple exchange pathways (both antiferromagnetic and ferromagnetic) can exist. Such a model has been set forth to explain the magnetic properties of the OEC assuming a tetranuclear assembly.¹²

As mentioned, the degree of magnetic exchange in multinuclear manganese clusters possessing biologically relevant ligands is relatively small owing to the predominantly ionic nature of the bonding. Therefore, when multiple oxidation states are present in these clusters the individual ions often retain some of their characteristic structural and coordination properties. A scheme for classification of mixed valence clusters has been presented by Robin and Day¹³ in which three classes are distinguished. "Class I" mixed valence compounds are completely valence localized systems in which the individual ions retain their characteristic bond lengths and distinct chemical properties. This lower limit of interaction is rarely realized in manganese complexes although some acetate bridged clusters come close. "Class II" clusters have centers that are crystallographically distinct but show significant similarities in the properties of the ions. This is by far the most common situation in biologically relevant manganese clusters and is most often seen for complexes with at least one single atom bridge.¹⁴ In these systems, intervalence charge transfer (ICT) excitations can often be observed at low energy (around $12,000\text{ cm}^{-1}$).¹⁵ Such a transition has been assigned to an ICT in photosystem II.¹⁶ The third class of mixed valence species having the extreme of complete electronic delocalization between metal centers is unrepresented in known biologically relevant manganese complexes.

EPR of Mononuclear Mn(IV) and Mixed Valence Dimers

The spin induced moment of nonintegral spin ions (Kramers ions) such as Mn(II) and Mn(IV) will interact with a magnetic field to give at least one accessible allowed transition in the electron paramagnetic resonance (EPR) spectrum. In contrast, the Mn(III) ion is generally EPR silent owing to rapid electron relaxation rates and larger zero field splitting, which separates the integral spin configurations.¹⁷ The EPR properties of Mn(II) are now well understood and have made this ion useful as an active site probe for numerous proteins.¹⁸ However, only recently have the properties of higher valent manganese been intensively studied. This has been done in an effort to understand the spectroscopy of enzymes now known to utilize these higher oxidation states.

The oxygen evolving complex displays an EPR signal at $g = 4.1$ under certain conditions. This resonance had been proposed to arise from a mononuclear Mn(IV)

ion¹⁹ or a conformationally perturbed manganese cluster²⁰. Although very recent studies suggest this feature arises from a manganese cluster²¹ with an $S = 5/2$ spin (see Chapter 8), an understanding of the spectroscopy of mononuclear $S = 3/2$ systems is still desirable. Until recently very few mononuclear Mn(IV) complexes of this type had been studied. A number of synthetic complexes have now been made²² and all of them show high field ($g = 4-5$) and low field ($g = 2$) signals (see Chapter 10 for new examples). The origin of the transitions giving rise to these signals has been determined and transition assignments have been made in the isoelectronic Cr(III) system.²³

The mononuclear manganese ion has primary energy contributions to the spin Hamiltonian arising from the electron Zeeman interaction, the electron spin-spin interaction (zero field splitting), and the nuclear hyperfine interaction. Nuclear quadrupole and nuclear Zeeman interactions usually yield only small perturbations on the energy levels and are generally not needed for an adequate description of the ion. Superhyperfine coupling to ligands is occasionally observed but due to the ionic nature of most manganese complexes, the electronic spin is primarily localized on the manganese ion and these couplings are small.²⁴ Nuclear hyperfine is quite often seen giving six distinct lines for the mononuclear manganese ion with $I = 5/2$.

For the octahedral mononuclear Mn(IV) ion, zero field effects split the $^4A_{3/2}$ ground state into two Kramer's doublets with effective spins of $1/2$ and $3/2$. Observed transitions are then confined to these zero field doublets and even in the event of large zero field splitting, the $S = 1/2$ spin manifold is still accessible. The zero field effects for Mn(IV) are generally large enough that the ion can be treated as a pseudospin $= 1/2$ system. For an axial system, this gives rise to effective g values of 4.0 (for g perpendicular) and $g = 2$ (for g parallel). As is shown in Chapter 10, this is exactly what is seen for powder samples of most known Mn(IV) monomers with the high field signal being more intense as expected. The shape of the high field signal is highly dependent on the electronic symmetry of the complex. From these studies and comparison to the Cr(III) systems it can be seen that the closer in energy the x and y components of the $M_S = -1/2$ to $M_S = 1/2$ transition, the more derivative the shape of the resultant high field signal will be. If a highly derivative low field signal originates from an $S = 3/2$ state of a cluster, the electronic symmetry of the multinuclear center must be very axial with x and y components nearly equal. Recent data indicating that the high field signal in the OEC arises from a multinuclear center will be discussed below.

Type II mixed valence manganese dimers also exhibit complex EPR signals often containing nuclear hyperfine contributions from each Mn center.¹⁶ Such signals have been observed for the S_2 state of the OEC as well as for the manganese catalase (see Fig. 1.1). In dimeric (or higher nuclearity) clusters where manganese ions share at least one bridging ligand, the Heisenberg exchange interaction dominates the electronic properties of the cluster. Dipolar interactions are usually small in these systems due to their $1/r^3$ drop-off with increasing Mn-Mn distance (r). The value of the Heisenberg exchange coupling constant (J) depends greatly on orbital overlap and the type of bridging ligand(s) mediating the superexchange.²⁵ The observed EPR spectrum for a dimeric system would be expected to be extremely complex if the