MANGANESE BIOCATALYSIS

Abstract

Manganese, a 3d transition metal, with variable oxidation states(-3 to +7) is ubiquitous in earth's crust, has low toxicity, and as a catalyst in cyanobacterial photosynthesis accumulated oxygen in the ocean which gradually escaped into the atmosphere displacing methane sometime between 2.4 – 2.1 billion years ago[1]. Cyanobacteria evolved from an anaerobic environment to produce oxygen which wiped out much of anaerobic life and gave rise to aerobic metabolism. Manganese thus plays a central role in evolution. Biophysical and biochemical studies of manganese as biocatalysts is described from a chemical and biological perspective in this chapter. The dissertation work done by the author dictates the choices in this chapter that are thus more like vignettes considering the vast amount of ongoing work in the area of Manganese bio catalysis. Manganese as a catalyst that produces hydrogen from water might turn out to be the key to a carbon neutral green economy of the future.

Keywords: Manganese, electron transport chain, Artificial Photosynthesis, redox

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I. INTRODUCTION

The electron transport chain (ETC) of mitochondria and chloroplasts are organelles that are similar in that they are both capable of synthesizing adenosine triphosphate (ATP), are made of protein complexes that receive and release electrons. Mitochondrial ETC uses chemical energy from redox reactions while chloroplasts use light energy. This chapter provides a review and comparison of the Manganese at the active site of two enzymes that is involved in photosynthesis as an electron donor that splits water to produce the oxygen we breathe and in mitochondria as part of an enzyme that accepts electrons from Oxygen superoxide radicals to prevent those radicals from causing damage. The results from dissertation[5] by this author are used because those results address a question that is different from looking at the intact in vivo catalytic site itself. Dissociation of the active preparation of PSII was achieved through high cationic and anionic washes. These treatments were characterized prior to the experiments that were carried out, however, the use of electron spin resonance spectrometry along with other biophysical tools permitted the examination and finding that it was possible to open up the manganese catalytic site (uncouple one of four manganese) and close it back again (couple all four manganese) through alternating treatments and examine the relative level of oxygen evolution, the activity catalyzed by Manganese. The results suggest that not all Manganese in the intact active site are probably essential for the activity though that contradicts the current models. This chapter will also review the Manganese in Superoxide Dismutase in the mitochondria where free radicals of oxygen are mopped up before they can cause damage by being converted to oxygen. Manganese thus plays arole in both oxidizing water to oxygen and reducing Oxygen radicals to oxygen. In one case it catalyzes by pushing electrons and in the other by pulling electrons from the substrate. Biologically, the superoxide anion $O_2^{\cdot -}$ and reactive oxygen species (ROS) play a dual role, a beneficial and a harmful one[37]. Current models are described as also other biophysical tools.

II. ETC IN MITOCHONDRIA(RESPIRATION) AND CHLOROPLASTS (PHOTOSYNTHESIS)

Both mitochondria and chloroplasts use an electron transport chain to generate ATP, but the processes differ in terms of location and input/output molecules. An electron transport chain (ETC) is a series of protein complexes located in the inner mitochondrial membrane (in eukaryotes) or the plasma membrane (in prokaryotes). It plays a crucial role in cellular respiration and photosynthesis, where it is responsible for transferring electrons and generating ATP (adenosine triphosphate), the energy currency of cells. In cellular respiration, the ETC is part of the aerobic respiration process. During the breakdown of glucose in the presence of oxygen, NADH (Nicotinamide adenine dinucleotide) and FADH2 (Flavin adenine dinucleotide) molecules are generated in earlier steps. These high-energy electron carriers donate their electrons to the ETC. The electron transport chain consists of several protein complexes, including NADH dehydrogenase, cytochrome b-c1 complex, cytochrome c, and cytochrome oxidase. These complexes are embedded within the mitochondrial or plasma membrane and contain specific electron carrier molecules such as flavin mononucleotide (FMN), iron-sulfur clusters, and heme groups. The flow of electrons through the ETC occurs in a sequential manner. Initially, NADH donates its electrons to the first complex of the ETC, often called Complex I. From there, the electrons are transferred to the subsequent complexes through a series of redox reactions, where electrons are shuttled between different electron carrier molecules. As the electrons move through the ETC, they

lose energy. This energy is used to actively transport protons (H+) across the mitochondrial or plasma membrane, creating an electrochemical gradient. This gradient drives ATP synthesis by a process called oxidative phosphorylation. At the end of the ETC, oxygen acts as the final electron acceptor, combining with protons to form water. This step ensures the continuation of the electron flow, as oxygen is a strong electron acceptor.

1. Mitochondria (Respiration)

- **Location:** The electron transport chain in mitochondria is located in the inner mitochondrial membrane.
- **Input:** The input molecules are reduced coenzymes, specifically NADH and FADH2, produced during glycolysis, the citric acid cycle, and fatty acid oxidation.
- **Output:** The output is ATP, which is produced through oxidative phosphorylation. Oxygen (O2) serves as the final electron acceptor, creating water (H2O) as a byproduct.

2. Chloroplasts (Photosynthesis)

- **Location:** The electron transport chain in photosynthesis is located in the thylakoid membrane of chloroplasts.
- **Input:** The input molecule is light energy absorbed by pigments such as chlorophyll. This energy is captured in the form of electrons in a molecule called chlorophyll
- **Output:** The output molecules are ATP and NADPH, which are used to power the synthesis of carbohydrates during the Calvin cycle (also known as the dark reactions of photosynthesis).

III. WATER OXIDATION: THE MOLECULAR COMPONENTS AND PROCESSES.

Four membrane spanning multi-subunit protein complexes present in the thylakoid membrane of chloroplasts work in concert to convert light energy into chemical energy[2]. In the late 1960s Murata identified what is termed as “state transitions” based on photochemical quenching observed in the kinetics of chlorophyll fluorescence[4]. The complex that acts as the antenna for light energy uses protein bound pigments that interact with two of the four complexes, namely, Photosystem I and Photosystem II. The functional identification of the protein complexes involved in the process is often restricted by what can be purified and isolated for study[3]. Fluorescence analyses indicate that the affinity of the light harvesting complex for PSI and PSII differ in affinity and the stoichiometry of the association is also different. The cytochrome b6f complex carries out a cyclic energy transfer such that part of the electrons transferred from PSII to PSI are cycled back to PSI. It must be noted that the thylakoid membrane provides the separation between the stroma and the inner compartment of the thylakoids as shown in Figure 1[5].

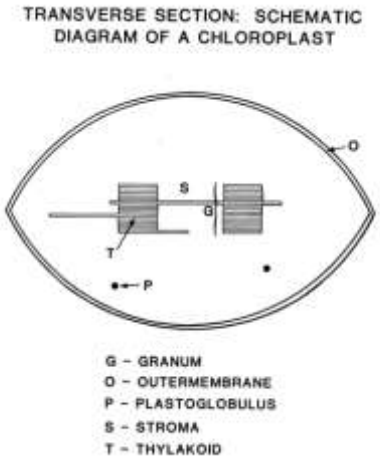

Figure 1: Transverse Section: Schematic Diagram of the chloroplast[5]

The separation between the stroma and the inner compartment of the thylakoids by the lipid bilayer creates the structure for charge separation. In the inner compartment or lumen of the thylakoid lie exposed through channels of water among the protein surfaces what is termed as the Reaction Center (RC). The RC is the location for four Manganese ions referred to as a cluster as they are held in place through covalent bonds that include bridging oxygen atoms to form a specific geometric pattern of a tetramer. Three of four manganese were found to be more tightly coupled as revealed through Electron Paramagnetic Resonance studies[5]. Furthermore, it was observed that calcium added to the medium was necessary to leach out one of the four manganese in preparations of photosystem II whose activity was measured using the oxygen electrode response to light the preparation was exposed to[5]. Reconstitution of this activity was attempted but it was found that the preparation that was depleted of one of four manganese was just as able to produce oxygen as the preparation that was not treated with Calcium and retained all four managanese[6,7]. The manganese cluster is the catalytic site for the oxidation of water to oxygen with a resultant charge separation when the protons and the oxygen are released to the lumen of the thylakoid while the electrons that are sourced from water are directed through the electron transport chain where Plastoquinone resides on the other side of the membrane, as described in the review[2]. The reaction can be represented as

Equation 1 $2H_2O+2PQ+4H+\text{stroma/cytoplasm}-\rightarrow h\nu O_2+2PQH_2+4H+\text{lumen}$

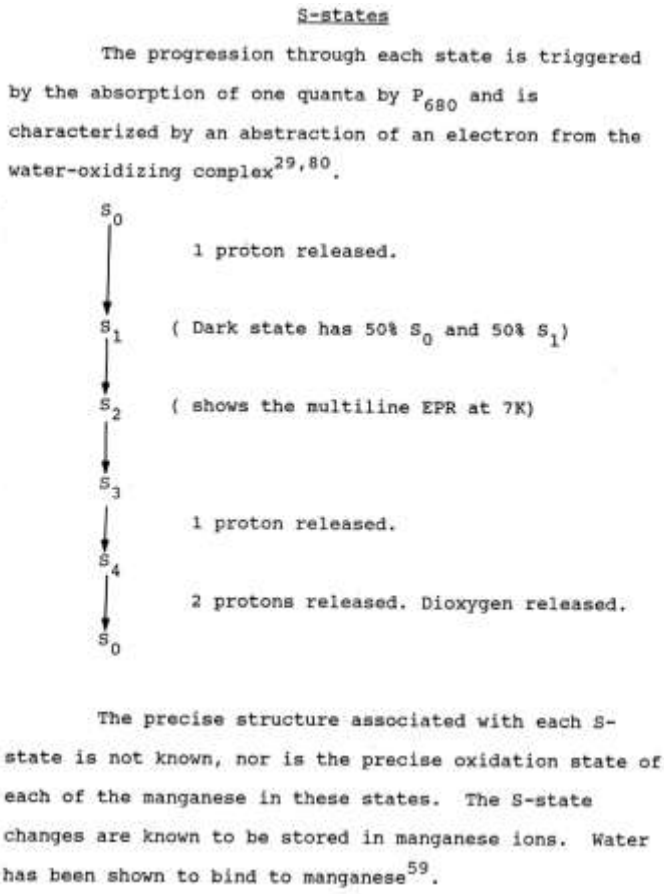

Figure 2: S-states of the oxygen-evolving complex (OEC)[5]

Figure 2[5] reproduced from the dissertation describes the use of Electron Paramagnetic Resonance (EPR) of Manganese to probe the functional organization of the manganese cluster. The findings published and cited in the dissertation suggest that a three manganese subcomplex suffices as the active site for water oxidation. This site was serendipitously found in the course of a set of investigations that sought to find porphyrin intermediates that could be trapped in a preparation of thylakoid membranes isolated from spinach that was at the time novel technique that had good degree of biochemical investigations of its composition. A calcium chloride wash of these membranes was found to be distinctly different in that it was characterized by an EPR signal which could be attenuated and recovered through cycles of low and high anion concentration in the medium for one. Though the Calcium chloride was known to remove three extrinsic proteins, the ability of these preparations to evolve oxygen as measured using the Clark electrode when illuminated with light, was found to be intact. Though therecently published model of a four manganese with Ca and 5 oxygen atoms model of the catalytic cluster[25] is well characterized, it does not necessarily imply that they are essential elements of the catalytic center.The model described[25] is based on intact preparations whereas the work in the dissertation[5] was based on treating the functionally active (Clarke oxygen electrode activity) preparations where the EPR signal would be attenuated but recoverable and found to be containing 3 Manganese coupled together whereas when the anions were added back, it would recover the signal characteristic of 1un-coupled Manganese. This cycling of the signal amplitude of the Q band EPR spectra when subjected to high chloride anions (2.0 M NaCl) and low chloride (35 mM

NaCl)is shown in Figure 3[5]. Calcium chloride washing of the preparation renders one of four manganese to be susceptible to EDTA extraction. This suggested that there were two pools of Manganese in the Photosystem II preparation, data not shown here, but described in the published work[5].

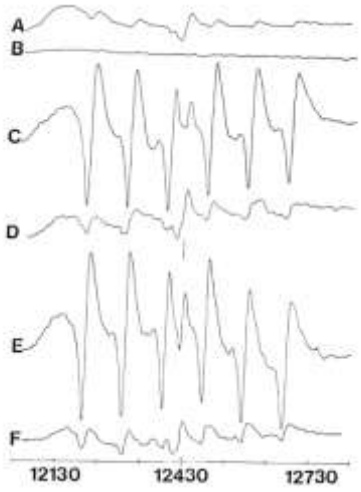

Figure 3A: Chloride depletion and re-addition cycles the signal intensity of the Q band EPR spectra[5]

The effect of chloride depletion and re-addition on $CaCl_2$-washed PS II preparations. Q-band EPR spectra of

A. untreated oxygen-evolving PS II membranes.

B. $CaCl_2$-washed PS II membranes.

C. sample B washed and resuspended in resuspension buffer (35 mM chloride).

D. sample C washed and resuspended in 2.0 M NaCl.

E. sample D washed and resuspended in resuspension buffer (35 mM chloride).

F. sample E washed and resuspended in 2.0 M NaCl.

Spectrometer sensitivity was essentially constant during all 6 runs. The small amount of protein-bound manganese visible in A can be completely removed by treatment with EDTA with no effect on the results of the subsequent treatments.

Figure 3B: Chloride depletion and re-addition cycles the signal intensity of theQband EPR spectra - key[5]

A model that explains the data shown in Figure 3A and 3B as also other data in the published work[5] is shown in Figure 4.

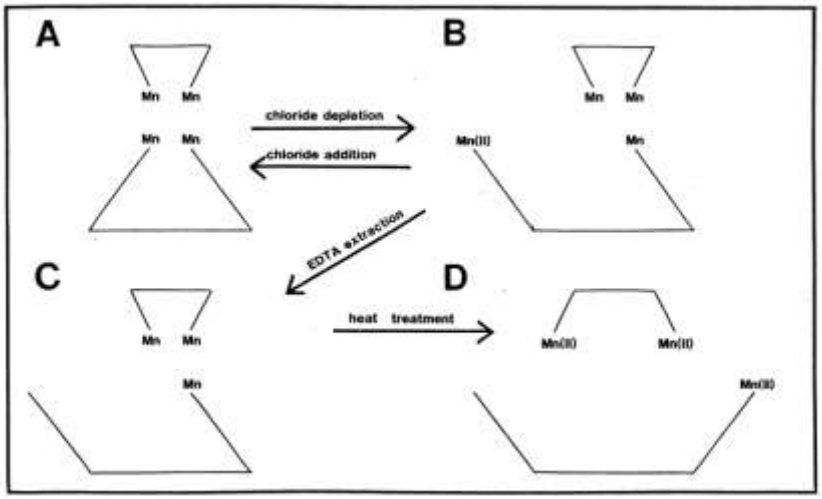

Figure 4: Model of the organization of manganese in PS II[5]

Panel C in Figure 3A corresponds to panel B in Figure 4, where the uncoupled Mn(II) results in the hyperfine structure of the G-Band EPR signal. EDTA treatment that results in the loss of 1 of 4 Manganese also results in the loss of extrinsic proteins (33 kDa and 17 kDa). To reconstitute biological activity in the absence of the leached Manganese, these proteins were added back to the preparation. Oxygen evolution measurements carried out on EDTA treated 3 Mn preparations to which the extrinsic proteins were added back, showed a recovery of oxygen evolution (27 % from 35%) as shown in Table 1A and 1B.

Table 1A: Recovery of steady-state oxygen evolution rates by reconstitution[5]

The recovery of steady-state oxygen evolution rates by the addition of extrinsic proteins to 4,3 and 2 Mn centers obtained from CaPSII membranes.

Sample type	# Mn / RC	O_2 evolution rate	%
Untreated	4.00 ± 0.01 (N=4, k=3)	463 ± 27 (N=4, k=2)	(100)
CaPSII	4.07 ± 0.03 (N=4, k=3)	28 ± 7 (N=4, k=2)	6
CaPSII + extrinsic proteins		163 ± 16 (N=5, k=2)	35
EDTA-washed CaPSII	3.04 ± 0.02 (N=4, k=3)	15 ± 5 (N=4, k=2)	3
EDTA-washed CaPSII + extrinsic proteins		124 ± 15 (N=4, k=2)	27
Mn-leached CaPSII + extrinsic proteins	1.96 ± 0.23 (N=1, k=3)	0 ± 0	0
Mn-leached CaPSII + extrinsic proteins	1.66 ± 0.11 (N=1, k=3)	22 ± 11	5
Mn-leached CaPSII + extrinsic proteins	2.56 ± 0.08	88 ± 20	19

contd.

Table 1B: Recovery of steady-state oxygen evolution rates by reconstitution[5]- key

N = # of sets, each set is from a preparation made on a different day ; k = the number of replicates within a set. n = N x k = total number of data points averaged. The average number of chlorophyll / reaction center was 292 + 8 from four preparations. All samples were in high salt buffer, on ice, in dark. Rate of oxygen evolution is micromoles O_2.(mg. Chl.)$^{-1}$.h^{-1} CaPSII membranes are PSII membranes that were washed twice with 1.0 M $CaCl_2$ and twice with high salt buffer (0.3 M sucrose, 0.2 M NaCl, 0.015 M $CaCl_2$, 0.05 M MES-NaOH, pH=6.0). EDTA-washed CaPSII were CaPSII washed twice in chloride free media, followed by a wash in 20 mM EDTA and a wash in chloride free media. This pellet was washed twice in high salt buffer. Mn-leaching was done by spinning down a sample of CaPSII kept in a chloride-free medium, on ice, after about 8 hours.

Three extrinsic proteins of 17, 23 and 33 kDa and a Manganese cover a three Manganese center at the water oxidation site. The EPR signal of such a preparation (untreated) shows a linewidth of 20 Gauss. This linewidth is unaffected by the removal of the 17 and 23 kDa extrinsic proteins (1 M NaCl). Removal of the three extrinsic proteins without removing Manganese can be accomplished by an alkaline 1.0M Tris wash (pH = 8.0). The evidence for retention of all 4 Manganese is supported by Atomic Absorption measurements and the unchanged linewidth of 20 Gauss in the EPR spectrum. When the preparation was subjected to divalent-salt washing with 1.0 M $CaCl_2$ all three extrinsic proteins are also released but the manganese is left intact. In the place of the broad 20 Gauss signal a narrower signal appears. Unlike the Tris treatment, the divalent salt wash renders the Manganese to become labile when the high salt is removed. This lability is reversible as it was found that the preparation could be cycled through low and high salt washes as shown in Figures 3A and 3B repeatedly. The labile Manganese is susceptible to extraction by EDTA as well. The uncoupled Manganese is shown in Figure 4 in panel B. The chloride addition and depletion cycle the state of the Manganese cluster between panels A and B as shown by the model in Figure 4. EDTA extracts only 1 of 4 Manganese.

IV. ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY

Free radicals, transition metal ions and defects in materials are materials with unpaired electrons. When exposed to a magnetic field these electrons orient themselves in the direction of the field and it leads to a split in energy levels of the population of these electrons which in turn increases as the magnetic field is increased. This is known as the Zeeman effect. In spectroscopy one could keep flip the population distribution across the quantized levels to produce a line. In EPR spectroscopy, the electromagnetic radiation is microwaves and their wavelength is not altered but the magnetic field in which the population of unpaired electrons are subjected to is changed. The theoretical line spectrum is not what

we observe but a spread across the field which is seen in Figure 3A as the width of the signal.

The intensity of the signal is also proportional to the number of unpaired electrons. A residual signal of low intensity can be found in untreated preparations. The EPR spectrum is the plot of microwave absorption (or derivative response) as a function of the magnetic field. The observed signal in this case is shown in the model as resulting from one of four manganese getting uncoupled from its original configuration where the coupling results in no signal. The uncoupled Manganese resides on a protein and the model in Figure 4B is a representation of a changed conformation that becomes possible only when the three extrinsic proteins are removed from the membrane protein complex of photosystem II by the Calcium chloride washing of the preparation. A similar wash with a mono cation like Sodium chloride or other halides (data discussed in the publication) does not render the Manganese labile enough to get uncoupled from the other three manganese. The model presented in the recent work[25] does show the asymmetry in the four-manganese catalytic center. A compact three Manganese core could be more biologically relevant is discussed in the review by Kusunoki[36].

V. FLAME ATOMIC ABSORPTION SPECTROMETRY

Manganese stoichiometry is determined using elemental analysis of the Manganese content relative to the Chlorophyll content, which is described in the literature cited in methods[5]. The chlorophyll content is the metric for the number of reaction centers present in the preparation. The number of Manganese determined per reaction center is reported as adjusted or normalized to the number of Chlorophylls per reaction center. Table 2 shows the data from this analysis

Table 2: Manganese in Photosystem II: Elemental Analysis by Flame Atomic Absorption Spectrometry[5]

Manganese in PSII : Elemental Analysis
by Flame Atomic Absorption Spectrometry

Set	# Mn / RC in CaPSII			# Chl./ RC
	Untreated PSII		EDTA-washed CaPSII	
1	4.00 ± 0.15	3.99 ± 0.15	3.06 ± 0.06	275
2	4.00 ± 0.09	4.10 ± 0.08	3.14 ± 0.25	311
3	4.00 ± 0.10	3.98 ± 0.02	3.00 ± 0.03	281
4	4.00 ± 0.11	4.29 ± 0.17	3.04 ± 0.17	307
Average	(4.00)	4.09 ± 0.09	3.06 ± 0.06	294 ±18

There were 3 replicates in each experimental set. Each set represents data from a preparation made on a different day. The standard deviation of the mean is reported for the average.

VI. POLAROGRAPHIC MEASUREMENT OF OXYGEN EVOLUTION

Oxygen evolution is assayed by a polarographic Clarke-type electrode using an artificial electron acceptor with a magnetic stirrer keeping the solution homogenous while shining light on it[26]. The potentiating voltage applied across a platinum cathode and silver anode results in a polarographic current generation at the cathode in the presence of oxygen which is amplified and recorded on a chart recorder. The scale of the recorder is calibrated in micromoles of oxygen. The slope of the light induced response is calculated and expressed as micromoles of oxygen evolved per mg. chlorophyll per hour.

VII. OTHER BIOPHYSICAL TOOLS

A general theory for analyzing the EPR spectra monomeric manganese (II) bound to proteins is presented by Meirovitch and Pupko[10].

1. **Transient Absorption (TA) spectroscopy:** TA Spectroscopy provides a window into the dynamic behavior of molecules and energy transfer processes following photoexcitation and is well suited for investigating fast processes on the picosecond to millisecond timescale. The technique uses a "Pump pulse", a short laser pulse, which induces transitions from the ground to the higher electronic states, creating a population of excited molecules. After a defined delay, a second "Probe pulse", a second laser pulse set to a particular wavelength to monitor specific transitions, or a broad spectrum is used. The probe light is absorbed by both the ground and excited state molecules. By comparing the intensity of the transmitted probe light to the intensity prior to excitation, the absorption changes are quantified. Furthermore, a systematic set of delays are applied between the pump and probe pulses to capture the temporal profile of the excited states. The use of this technique to study has been enhanced through the development of a series of software tools to perform global, lifetime and target analysis of in vivo datasets[27]. When used in conjunction with stopped flow techniques, time resolved spectroscopy has been used to examine the structure-function relationships of redox mediators[28].

2. **ELDOR / DEER:** Angstrom level resolution of the distance between unpaired electron spins is measured using ELDOR/DEER which measures the dipolar coupling between the spins. Electron-electron double resonance (ELDOR) also known as double electron-electron resonance (DEER) is a technique that is often used in conjunction with electron paramagnetic resonance (EPR) spectroscopy. A simplified outline of how it works: 1. reparation step: The sample is placed in a magnetic field to align the spin states of the electrons. The magnetic field causes energy gaps between the spin states to match the energy of the microwave radiation, or in other words they are said to be in "resonance" with the microwaves. 2. First Pulse: An initial microwave pulse is applied that flips the spin states of the electrons. 3. Second Pulse: After a certain delay, a second pulse is applied. The second pulse is applied at a different frequency than the first to perturb it to a different electron spin center. This introduces a phase shift in some of the spins, creating an imbalance, which in turn establishes an observable echo. 4. Third Pulse: After another delay, a third pulse identical to the first pulse is applied to all the spins, which leads to the formation of a spin echo. 5. Observation: The resultant echo signal decay is detected as a function of time, after the third pulse. This signal, known as the DEER echo, is analyzed to get information about the distances between the two different types of spin systems. This makes the technique well suited to detect conformational

changes in proteins bearing paramagnetic centers. Models based on such studies have been reviewed recently[29].

3. **NMR microscopy and reflectance spectroscopy:** NMR microscopy and reflectance spectroscopy on whole leaves was used to determine chloroplast water content[30]. The nuclei of hydrogen in water yields a strong nuclear magnetic resonance (NMR) signal. This signal can be spatially mapped, resulting in a representation of where water is located within a leaf. The study reports the relative water content between the water in the chloroplasts and the other cellular components of the leaf. The chlorophyll in the leaf strongly absorbs light in the blue (440-470 nm) and red (640-680 nm) regions and reflects more in the green (550 nm) region, which is why leaves appear green. Reflectance spectroscopy measures the amount of light that reflects off a surface, as opposed to the amount of light that is absorbed or transmitted. A spectrophotometer is used to measure reflected light. Leaves with more water generally reflect more light in the near-infrared region and this can be used in remote sensing to assess crop health much ahead of time before crop sustain damage. SCF-MO calculations were used to obtain the principal components for the g-tensor of hydrogen and fluorine peroxy radicals to develop a general theory that can be used to analyze the ESR spectra to chemically identify peroxy radicals[31].

4. **Oxygen K-edge X-ray Absorption Spectroscopy (XAS):** This is a powerful tool to determine the electronic structure of oxygen-containing materials and environments in chemistry and biology. XAS operates by focusing high energy X-rays onto a sample. These X rays have enough energy to eject core electrons (in this case the 1s electrons from oxygen atoms) from their atomic orbitals. This causes them to be excited into unoccupied states or get completely ejected from the atom. The “K-edge” refers to the energy at which the 1s electron are excited. Oxygen K edge XAS measures how the ability of a sample to absorb X rays changes as the energy of the x rays is tuned. As the energy approaches the binding energy of the 1s electrons of Oxygen (“K edge), the absorption spikes in the spectrum. The resulting spectra can tell us a lot about the unoccupied electronic states that the 1s electron are being excited into, or in other words reveals the local electronic structure around the oxygen atom. It reveals if the oxygen is in a hydroxyl group (-OH), an oxide (O2-), or bonded with other elements. The method finds applications in diverse fields such as material science, chemistry, geology, environmental science and biology. The method has been given different names based on some variations such as XANES (X ray absorption near edge structure) or NEXAFS (near edge X-ray absorption fine structure), ELNES (near edge energy loss spectroscopy), IXS (inelastic x ray scattering), XRS (X ray Raman scattering) or NIXS (non-resonant inelastic X-ray scattering). Transition metal oxides such as Manganese coupled with oxygen as discussed in this chapter, is therefore a special case of applying this technique. The method is reviewed for various oxides[33]. The ability of Manganese to draw electrons away from the oxygen in Mn-O bonds are crucial for the formation of oxygen from water[34].

VIII. ARTIFICIAL PHOTOSYNTHESIS

Artificial Photosynthesis is a promising route to achieving carbon neutral energy sources. Solar driven water oxidation that generates hydrogen ions in a bionic leaf is coupled with a bioengineered bacterium to convert carbon dioxide from air in a report by Dogutan

and Nocera[12].The hurdles to inexpensive, durable heterogenous catalyst for mimicking water oxidation is due to an insufficient understanding of the reaction mechanisms in a review by Zhang et. al.[13]. The reaction rates at which electron transfer occurs between the light harvesting complexes (LHC1 and LHC2) and the reaction center (RC) pigments is in the order of pico to femto seconds range. As the last step is the slowest (pico seconds versus femto seconds) it prevents back reactions. McConnell, Li and Brudvig provide a review of natural and artificial photosynthesis[14]. Zhang and Sun[15] provide a review of the opportunities and challenges of molecular catalysts. The catalytic turnover rate (TON) and turnover frequency (TOF) are parameters that define a good water oxidation catalyst. In nature Water oxidation occurs at a low overpotential (approximately 160 mV) and a high reaction rate (100 – 400 per second). The actual mechanism of water oxidation in Photosynthesis, as to how the O-O bond formation occurs is yet to be determined and there are two candidate mechanisms that are proposed and lack experimental verification. The candidate mechanisms are a water nucleophilic attack (WNA) and interaction between two M-O intermediate (I2M) pathways to form O-O bonds (oxygen evolution) catalyzed by molecular catalysts. The first molecular water oxidation catalyst (WOC) reported in the 1980s was Ru based, the so-called blue dimer (BD). It had a TON of 13 and TOF of 0.0042 per second. It was close to three decades before a breakthrough was seen with a molecular WOC with a TON of 2000 and a TOF of 41 per second. This breakthrough was accomplished by using carboxylate groups into the ligands and a special steric configuration that allowed 7-coordination at the at the catalytic site. The overpotential was reduced from 370 mV to 180 mV and the TON changed from 0.004 per second to 41 per second and a change in TON from 13 to 2000[15]. This change was inspired by the OEC in PSII containing several carboxylate ligands. Among the challenges to overcome are that current WOCs are noble metal Ruthenium based and not the earth abundant metals. Low stability, high cost and moderate light absorption limits commercialization of the technology. A model compound that mimics the biology of the water oxidation site catalytic center was able to protect the catalytic site from the side reaction by binding it to a TiO_2 surface using a chromophoric linker[32]. The solid-state structure of Manganese oxides plays a key role in their efficiency as catalyst for water oxidation with the most promising structural motif being large tunnels which are reminiscent of proteins with channels and pores[35]. Computational approaches to guide and interpret experiments that could also lead to generating data that could be used by Machine Learning to further guide the design of artificial photosynthesis is reviewed in a Nature briefing[38]. Metal nanoparticles (NPs) could be analogous to the protein residues controlling the metal bound biocatalytic center, that could be tailored and characterized. The synthetic methods to produce such metal nanoparticles are discussed in the review with a comparison to the parameters for artificial photosynthesis[39].

IX. MANGANESE IN SUPEROXIDE DISMUTASE

Reactive oxygen species (ROS) result from the ETC in mitochondria. Dismutation is a chemical reaction in which a single compound is simultaneously oxidized and reduced, resulting in the formation of two different products. In the context of Superoxide dismutase (SOD) enzymes, dismutation refers to the enzymatic reaction in which superoxide radicals (O2-) (ROS) are converted into oxygen (O2) and hydrogen peroxide (H2O2). superoxide (O2-) is dismutated into molecular oxygen (O2) through oxidation, and hydrogen peroxide (H2O2) is formed through reduction. Besides mitochondria, SODs, discovered in 1968 use other transition metals and have a wide distribution in other tissues as well with a similar protective function that plays an important role in a wide range of disease conditions such as

cancer of the colon lung and lymphatic system as well as neurodegenerative diseases. Heart and stroke patients often succumb to the cell damage and death that occurs after blood and oxygen are restored to ischemic or hypoxic tissue, whose treatment is using organometallics is described in a perspective[24]. The human Mn-SOD (manganese superoxide dismutase) enzyme is a homotetramer, meaning it consists of four identical subunits, each containing a manganese metal ion as a cofactor. The structure of human Mn-SOD enzyme can be described as follows:

1. **Subunit Structure:** Each subunit of the Mn-SOD enzyme consists of a single polypeptide chain folded into a compact globular structure. The polypeptide chain comprises approximately 200 amino acid residues. The four subunits come together to form the complete Mn-SOD enzyme.

2. **Active Site:** The active site of Mn-SOD, where the manganese ion is located, is situated in the interior of each subunit. The manganese metal ion is coordinated by amino acid residues of the protein, forming the catalytic center. The coordination environment surrounding the manganese ion helps facilitate the dismutation reaction of superoxide radicals.The manganese cluster typically consists of two manganese ions, often referred to as the "redox-active" manganese (Mn3+) and the "catalytic" manganese (Mn2+), which are bridged by amino acid residues. The exact amino acid residues involved can vary, but commonly, they include histidine and aspartic acid residues.These manganese ions are often bridged by oxygen-containing ligands, which can include water molecules or amino acid side chains. These bridges stabilize the cluster and influence the reactivity of the manganese ions.The MnSOD active site coordinates with the superoxide substrate, allowing the manganese ions to participate in the dismutation reaction. The manganese cluster plays a role in cycling between different oxidation states, enabling the enzyme to catalyze the conversion of superoxide radicals. The detailed mechanism involves the formation of a manganese-peroxide intermediate that leads to the production of molecular oxygen and hydrogen peroxide.The manganese cluster is strategically positioned within the active site of MnSOD to accommodate the superoxide substrate. The binding and orientation of the superoxide molecule relative to the manganese ions are critical for the catalytic process. The catalytic mechanism of the manganese cluster in human MnSOD is interesting in how unlike other Superoxide Dismutases, the one in the mitochondria where the electron transport chain is similar to that in the chloroplast uses Manganese whereas Nickel and Iron is often the catalyst in prokaryotes[23].

3. **Secondary Structure:** The protein chain of Mn-SOD contains various secondary structures including alpha helices and beta strands. These structural elements provide stability and contribute to the overall folding of the enzyme.

4. **Quaternary Structure:** The homotetramer assembly of Mn-SOD is stabilized by non-covalent interactions between the individual subunits. These interactions can involve hydrogen bonding, ionic interactions, van der Waals forces, and hydrophobic interactions. The quaternary structure of Mn-SOD is crucial for its stability and enzymatic activity. The precise arrangement and three-dimensional structure of human Mn-SOD have been determined using techniques such as X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy. These methods have allowed scientists to visualize and understand the detailed structure and coordination of the manganese cofactor within the Mn-SOD enzyme.

5. **Biology:** In the course of metabolic processes especially in aerobic (oxygen utilizing) organisms, an oxygen molecule acquires one (unpaired) electron to form a superoxide molecule which is highly reactive and is known to cause damage that is implicated in a wide array of diseasesand conditions, including aging, cancer, cardiovascular diseases, neurodegenerative disorders, and inflammation. Nature has evolved defense mechanisms that involve antioxidant enzymes such as superoxide dismutase (SOD), catalase, and various non-enzymatic molecules like vitamin C, vitamin E, and glutathione. These antioxidants help neutralize super oxides by converting them into less harmful molecules and maintaining cellular redox balance.

6. **SODs:** Superoxide Dismutases are metalloenzymes that are differentiated by the redox active metal copper (Cu/Zn SOD), MnSOD or FeSOD and NiSOD that form three evolutionary families. The FeSODs are found in prokaryotes and MnSODs in the mitochondria near the electron transport chain.

7. **MnSOD mimetics:** Recombinant SOD enzymes have been used in some preclinical trials to be effective therapeutic agents, however, being proteins, their scope is very limited. Therefore, non-proteinaceous synthetic low molecular weight mimetics of the SOD enzymes have found wide adoption as pharmaceutical agents to treat various superoxide-related ailments. Important aspects of chemistry, in particular, redox activity, and biochemistry of superoxide and nitric oxide as well as their interaction with MnSOD mimetics that should be considered for the future design and pharmacological studies of manganese-based therapeutics and even diagnostic tools are covered in the review by Ivanovic-Burmazovic and Filipovic[16]. Since superoxide solutions are unstable due to the fast reaction, characterizing SOD activity of mimetics requires careful assessment. Pulse radiolysis and stopped flow measurements are direct methods that follow the decomposition of the superoxide in the UV region [17-19]. Indirect methods (variations of the cytochrome c or NBT/MTS/XTT assays) track the inhibition by the putative SOD mimetic of the reaction between an indicator and in situ generated (usually by xanthine/xanthine oxidase system or 60Co gamma irradiation) superoxide[20-21]. A review of the catalytic activity of synthetic Manganese complexes provides insights into catalysis design[22].

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