A Biomimetic Manganese Model for Artificial Photosynthesis

Q-band Electron Paramagnetic Resonance Study of a Novel Unsymmetric Mn$_2$(II,III) Complex

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The Master’s Thesis is submitted to Linköping Institute of Technology, Sweden, in acquiring Master’s of Science in Technical Biology. The study was conducted at the Consortium of Artificial Photosynthesis, Uppsala University, with the supervision of Docent Ann Magnuson during September 2004 and February 2005. The thesis is equivalent to 20 weeks of full time studies.
ABSTRACT

In natural oxygen-producing photosynthesis solar energy is stored as chemical energy, in carbohydrates, fats and amino acids, using water as electron source. The large transmembrane protein complex, PSII, is the key enzyme in the light-driven reactions. Water oxidation is accomplished by a triad in PSII in which the Mn-cluster plays an important role. In the artificial photosynthetic system, nature’s photosynthesis will be mimicked such that hydrogen, a sustainable energy source, can be produced from solar energy and water alone. Since water oxidation requires the catalytic activity of a Mn-cluster in photosynthesis, different artificially constructed manganese complexes are investigated.

The dinuclear \([\text{Mn}_2(\text{II,III})L(\mu\text{-OAc})_2]\text{ClO}_4\), where \(L\) is the \(X\)-anion of \(2-(N,N\text{-Bis}(2\text{-methylpyridyl})\text{aminomethyl})-6-(N-(3,5\text{-di-tert-butylbenzyl-2-hydroxy})\text{-N}(\text{pyridylmethyl})\text{aminomethyl})\text{-4-methylphenol}\), an unsymmetric ligand with two coordinating phenolate groups, has been studied. The two Mn-ions are linked via a mono-\(\mu\)-oxo bridge and two acetate ligands. Q-band Electron Paramagnetic Resonance was conducted on the Unsymmetric \(\text{Mn}_2(\text{II,III})\) Complex. Acquired results show that the complex has a 2600 Gauss broad signal (11 400-14 000 Gauss) with 14-17 lines at \(g\sim2\) and hyperfines of 120 Gauss. This is consistent with previous X-band studies. Q-band spectra of the Unsymmetric \(\text{Mn}_2(\text{II,III})\) display increased hyperfine resolution compared to Q-band spectra of the symmetric complex, \(\text{Mn}_2(\text{bpmp})(\mu\text{-OAC})_2\). This is noticeable since Unsymmetric \(\text{Mn}_2(\text{II,III})\) and \(\text{Mn}_2(\text{bpmp})(\mu\text{-OAC})_2\) partly overlap in low-frequency experiments (X-band EPR).

Further investigations are yet to be expected. Nevertheless, the conducted thesis study provides important knowledge in the futuristic goal of building an artificial super-complex.

Keywords: Photosynthesis, manganese, electron paramagnetic resonance
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<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>Unsymmetric Mn$_2$(II,III)</td>
<td>Unsymmetric Manganese Complex</td>
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<tr>
<td>Mn$_2$(bpmp)(µ-OAC)$_2$</td>
<td>Symmetric Manganese Complex</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid (2'H)</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid (2'OH)</td>
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<tr>
<td>PSII, PSI</td>
<td>Photosystem II, Photosystem I</td>
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<tr>
<td>Ca</td>
<td>Calcium</td>
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<td>$P_{680}$</td>
<td>Primary donor chlorophylls of PSII (absorption maximum 680 nm)</td>
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<tr>
<td>$P_{700}$</td>
<td>Primary donor chlorophylls of PSI (absorption maximum 700 nm)</td>
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<td>$P_{680}^+$</td>
<td>Photo-oxidized $P_{680}^+$</td>
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<tr>
<td>Tyr$_z$, Y$_z$</td>
<td>Tyrosine amino acid (Tyr on D1 subunit of PSII)</td>
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<td>Q</td>
<td>Quinone</td>
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<tr>
<td>EPR</td>
<td>Electron Paramagnetic Resonance</td>
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<tr>
<td>OEC</td>
<td>Oxygen Evolving Complex</td>
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<tr>
<td>Mn</td>
<td>Manganese</td>
</tr>
<tr>
<td>Mn$_4$</td>
<td>Cluster of four manganese ions</td>
</tr>
<tr>
<td>Ru</td>
<td>Ruthenium</td>
</tr>
<tr>
<td>Mn(II)</td>
<td>Mn$^{2+}$</td>
</tr>
<tr>
<td>Mn(II,III)</td>
<td>Mn$^{2+}$Mn$^{3+}$</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>NADP</td>
<td>Nicotinamide adenine nucleotide phosphate</td>
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<tr>
<td>Ru(bpy)$_3$</td>
<td>Ruthenium(II)-tris-bipyridine (Photosentisizer)</td>
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<td>NDI</td>
<td>Naphthalene diimide (Acceptor)</td>
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1 INTRODUCTION

1.1 Background

Photosynthesis, an ancient process that gradually accumulated oxygen in the Earth’s atmosphere, involves green plants, cyanobacteria, photosynthetic bacteria and algae [1]. These organisms take advantage of natural resources as sunlight and water in order to make energy rich carbohydrates for their survival and growth, as well as providing material for non-photosynthetic organisms. Even the modern industry utilizes the products of photosynthesis, such as fossil fuels (coal, oil and gas) are all remains of archaic organisms. Unfortunately, the use of fossil fuels have led to higher concentrations of CO$_2$ in the atmosphere. CO$_2$ being one of the gases contributing to global warming. Additionally, an increasing depletion of nature’s reservoir is evident. The ultimate goal for our energy consumption must thus be to use sustainable resources that do not have negative environmental contribution. We need to think ahead and propose solutions that will hold in the future. In other words, even if there is an exponentially increasing energy demand, we have a responsibility toward the next generations to try to solve these problems.

1.2 The Dream of An Artificial Photoynthesis Scientist

– A Potential Solution

Water is by far one of the most important resources on earth since it is abundant, cheap and environmentally clean. The plants use water as an electron source contributing to their large dominancy in nature. In order to oxidize molecular water much energy is required. If the green plants manage to accomplish this phenomenon by having only sunlight as a sufficient energy source, then this process would be interesting enough to be investigated. Solar energy can be used directly in areas where the sun shines abundantly. Though, in countries like Sweden where there is less sunlight, this approach would be limited. Therefore an idea came to birth which would enable an indirect utilization of solar energy.

Artificial photosynthesis is based on mimicking nature’s photosynthesis in order to convert light energy to chemical energy. Different ideas are under development, some are discussed in this thesis. Construction of super-molecular complexes (linkage of catalytic and redox active molecules) would be an alternative solution enabling a photo-induced intramolecular electron transfer, similar as the natural photosynthesis. The electrons gained by oxidizing water into oxygen are used to produce an energy rich fuel such as hydrogen. Environmentally speaking, as water is the only waste product, hydrogen is an attractive fuel in the future, even if some problems with storage facility (hydrogen is an explosive gas) and chemical reactivity are present. These problems have though already been improved.
1.3 The Aim of the Thesis

Artificial Photosynthesis is a broad research field with numerous issues to be addressed and perhaps solved. Considering the effectiveness of the water oxidizing complex in natural photosynthetic systems, there has been a main focus on the catalytic activity of the manganese ion cluster. Therefore several artificial manganese complexes have been synthesized and characterized thoroughly. Recently a novel unsymmetric dinuclear manganese complex was successfully constructed. The aim of this study has been to investigate the properties of this complex in a mixed valence state system (a manganese dimer in (II,III) valence state), which hopefully will be used for constructing artificial photosynthetic super-complexes. The technique of choice has been high-frequency electron paramagnetic resonance, EPR.
2 PHOTOSYNTHESIS

2.1 Introduction

Green plants, algae and cyanobacteria all utilize light energy and atmospheric CO$_2$ to synthesize energy rich products in a process called photosynthesis [1]. Higher forms of plants began to exist when cyanobacteria gradually accumulated oxygen gas in the atmosphere. Photosynthesis is based on the conversion of light energy into chemical energy by series of photosynthetic reactions. The process would not be possible without the presence of some specific pigments, which can capture and package the sun light for further chemical reactions. One of these important pigments is chlorophyll which can absorb light at various wavelengths (400-700 nm). Photosynthesis is a very complex reaction in which the overall scheme is:

\[ n \text{CO}_2 + n \text{H}_2\text{O} \xrightarrow{\text{light}} (\text{CH}_2\text{O})_n + n \text{O}_2 \]

(CH$_2$O) is the building block for all carbohydrates such as sugars and starch (n represents an integer).

2.2 Organisation

In green plants, the site of photosynthesis occurs in the chloroplast organelle. These are present in numerous quantities in the cell and each chloroplast is bound by a highly permeable outer membrane and a nearly impermeable inner membrane in which they both are separated by an inter membrane compartment. Enclosed by the inner membrane is the stroma, a concentrated solution which consists of enzymes necessary for the conversion of CO$_2$ to carbohydrates. The stroma also contains the chloroplast’s own DNA, RNA and ribosomes for the synthesis of chloroplast proteins. A third membrane is suspended in the stroma as the thylakoid membrane, an extensive internal network. The dark reactions occur in the thylakoids and the light driven reactions in the stroma. Overall the light reactions consist of photochemical reactions, electron transport, oxygen evolution, ATP synthesis and NADP$^+$ reduction, while the dark reactions are driven by ATP and NADPH dependant CO$_2$ fixation and other biochemical reactions [1].

Figure 1. The chloroplast organelle consists of three membrane layers; the outer, the inner and thylakoid membrane [1].
CHAPTER 2. PHOTOSYNTHESIS

2.3 The Photosystems - PSII and PSI

All photosystems in green plants are constituted of three major components. The reaction center, where the photochemical charge separation occurs, the inner antennas and the peripheral antennas consisting of several chlorophyll molecules that guide the light to the reaction center. Energy-transfer pathway starts from the peripheral antennas, continues towards the inner antennas before reaching the final destination, the reaction center [1].

In the light driven reactions, PSI, PSII and different electron carriers, are responsible of converting CO$_2$ into energy rich products by accumulating reducing power as NADPH and ATP, generated by the proton gradient. Both NADPH and ATP are important factors in the biosynthesis of carbohydrates, fats, amino acids etc. Each of the protein complexes, PSI and PSII, absorbs light at different wavelengths. They are surrounded by light harvesting chlorophyll molecules in order to contribute an effective energy transfer to their reaction centers [1].

2.3.1 Photosystem II

In green plants, algae and cyanobacteria, PSII oxidize water into molecular oxygen, electrons and protons [1].

$$H_2O \rightarrow \frac{1}{2}O_2 + 2H^+ + 2e^-$$

Figure 2. (A) The X-ray structure of two PSII subunits, D1 (dark green) and D2 (purple), and their mutual interaction at 3.7 Å (Ångstrom). The structure contains four chlorophylls (light green), two pheophytins (blue), Tyr$_z$ (yellow) on D1 and a cluster of four manganese ions (red). (B) The arrangement of the chlorophylls and the PSII cofactors. Viewed along the membrane plane [2].
PSII consists of over twenty subunits, most of them embedded in the thylakoid membrane. The major protein complex in the reaction center is a heterodimer of two subunits, D1 and D2. Together these contain the cofactors and the pigment molecules involved in the electron transport. The primary donor of PSII, \( P_{680} \), is associated to the two subunits. In order for PSII to achieve water splitting, a manganese/calcium ion cluster, \( \text{Mn}_4/\text{Ca}^{2+} \), with four manganese atoms and a tyrosine residue, \( Y_z \) on the D1 subunit, are required [3]. When light shines on PSII, \( P_{680} \) becomes excited and donates electrons to components on the acceptor side of the electron transport chain. \( P_{680} \) is then re-reduced, through \( Y_z \), by the \( \text{Mn}_4/\text{Ca}^{2+} \) cluster. Water splitting is accomplished by exploiting the different valence states of the manganese ions (see section 4.2) [3].

### 2.4 Electron Transfer in the Thylakoid Membrane

The reactions of PSII involve a light-driven primary and secondary electron transfer process across the reaction center in D1 and D2 subunits. The photochemistry of PSII is initiated by light excitation at 680 nm, where the absorption peak is at its maximum. Upon illumination the primary donor chlorophylls \( P_{680} \) become excited and release an electron to pheophytin and subsequently to the membrane-bound quinone \( Q_A \). The electron released by the oxidized \( P_{680}^+ \) is transferred to the final electron acceptor \( Q_B \), which takes up two protons from the stromal side and becomes reduced. This releases plastoquinol, PQH\(_2\), into the membrane and it is replaced by oxidized plastoquinone from the quinone pool in the membrane. Reduction of oxidized \( P_{680}^+ \) is accompanied by the redox-active tyrosine residue \( Y_z \), which stabilizes the charge-separated state. The neutral radical \( Y_z^- \) in turn oxidizes the tetranuclear manganese cluster through the different \( S_n \)-states (\( n = 0-4 \)) in the S-cycle (Kok’s cycle, section 4.2) where oxidizing equivalents are stored in order to split water as a final step [4]. PSI itself undergoes light-excitations at 700 nm, followed by electron donation in several steps for the reduction of NADP\(^+\) to NADPH. \( P_{700}^+ \) is re-reduced by the transfer of electrons from \( P_{680}^+ \) through the quinone pool, cytochrome b\(_{6f}\) and plastocyanin [3].

![Figure 3. A schematic representation of the electron transport chain in the thylakoid membrane. From the photo-excitation of \( P_{680} \) to ATP- production by chemiosmosis [1].](image-url)
3 THE ARTIFICIAL PHOTOSYNTHESIS MODEL

3.1 Introduction

Since PSII is the only known chemical catalyst for splitting water, it is important to understand the mechanisms at atomic levels. The ability of PSII to extract electrons from water is the main reason why plants are the dominating life on earth. Where there is sun and water there is always life.

Energy created in the light-driven reactions of plants is used for the synthesis of carbohydrates in the dark-driven reactions. The artificial research desires to mimic the light-driven reactions which in the natural system accounts for up to 40-50 % efficiency of the absorbed sunlight [5]. When using hydrogen as an alternative energy carrier (since electric energy can not be stored), the same proportion in efficiency is reached. Furthermore, hydrogen is an environmentally clean fuel, creating water as the only waste product. As storage and transportation of hydrogen are widely advanced today, it creates a positive outlook for developing a hydrogen source in the future. Not surprisingly, understanding the plant’s PSII will help provide valuable information towards this goal.

3.2 The Consortium of Artificial Photosynthesis

The Consortium of Artificial Photosynthesis was founded 1994 in Sweden, and the organisation consists currently of five research groups:

1. The Biomimetics group (Professor Stenbjörn Styring, Uppsala).
2. The Physical Chemistry group (Professor Leif Hammarström, Uppsala).
3. The Femtosecond group (Professor Villy Sundström, Lund).
4. The Organic Synthesis group (Professor Björn Åkermark, Professor Licheng Sun, Stockholm).
5. The Biohydrogen Production group (Professor Peter Lindblad, Uppsala).

In total the Consortium includes around 40 people, not accounting for the continuous collaboration with several groups around the world. Except for the Swedish Artificial Photosynthesis research that focuses mainly on constructing supramolecules to produce fuel from sunlight and water, other groups create molecular systems similar to the natural photosynthesis which are not energy related. These groups are found in USA, Germany, France, Japan, Italy and Ireland.
3.3 Natural Photosynthesis vs Artificial Photosynthesis

There are three basic components in natural photosynthesis that are necessary in order to split water [6]:

1. A photon absorbing molecule capable of light-excitation; chlorophyll
2. An electron carrier that mediates electrons to the chlorophyll; tyrosine residue
3. A catalyst that splits water and transports electrons to the chlorophyll via tyrosine; manganese complex

![Diagram of natural photosynthesis]

The artificial photosynthesis research is transferring each individual component into a synthetic network that in principal will be able to accomplish the same goal. With some modifications from the natural photosynthesis, the results will hopefully lead to the water oxidation. This is the proposed model for the different key parts [6]:

1. A light-harvesting complex; ruthenium metal
2. An electron mediator; also a tyrosine amino residue
3. A water binding complex that carries catalytic activity; also a manganese complex

The artificial model includes a photosensitizer that undergoes light excitation, a donor molecule that splits water and donates electrons to the photosensitizer, and an acceptor molecule that receives electrons from the photosensitizer. These molecules must be able to perform the reactions collectively in an effective manner.
CHAPTER 3. THE ARTIFICIAL PHOTOSYNTHESIS MODEL

3.3.1 The Key Factors of the Super-Complex

Each individual factor, the donor, the photosentisizer and the acceptor, is coupled to a super-complex, with the ability to oxidize water through intramolecular electron transfer. However, the crucial problem lies in extracting enough electrons from the manganese complex in order to oxidize water.

3.3.1.1 The Donor

Today the donor molecule is suspected to be a complex of manganese ions that can accumulate redox equivalents and split water as well as donate electrons via a tyrosine amino acid residue to the photosentisizer. At this early stage in the artificial photosynthesis research, for practical reasons, complexes of dinuclear manganese are investigated instead of tetranuclear manganese as in nature.
3.3.1.2 The Photosentisizer
Ruthenium(II)-tris-pyridine, Ru(bpy)$_3$, proves to be a good molecule that absorbs light in wide absorbance intervals and is stable in base and acid solutions. Ru(bpy)$_3$ has the similar properties as the chlorophyll molecule; it undergoes photo-induced oxidation and has enough oxidation potential, $\text{Ru}^{2+/3+} = 1.23 \text{ V (P}_680/\text{P}_{680}^+ \sim 1.12 \text{ V})$. The molecular properties of Ru(bpy)$_3$ are such that it is relatively easy to chemically built adjacent molecules to it. The ruthenium complex is more robust than the chlorophyll thereby reducing its sensitivity to light. In plants the chlorophyll sensitivity is compensated by different recovery systems.

3.3.1.3 The Acceptor
In preliminary studies the acceptor has been a cobolt complex that upon reduction changes its valence state from Co(III) to Co(II). Recently the acceptor NDI (naphthalene diimide) has also proved to complete a photo-induced intramolecular electron transfer with a long-lived charge-separation state similar to the PSII donor side. The ultimate goal for the coupled electron accepor is the ability to reduce protons to hydrogen.

3.3.2 Synthetic Manganese Complexes
Certain properties of the manganese cluster have to be accomplished in order to obtain water oxidation. Early studies of various artificial manganese complexes resulted in successful extractions of electrons. As discussed earlier, water oxidation into its components requires not only one electron abstraction, but rather four.

The symmetric manganese complex in 1, Mn$_2$(bpmp)(µ-OAC)$_2$, with the ligand bpmp (the anion of 2,6-bis{[N,N-di(2-pyridinemethyl)amino]methyl}-4-methylphenol) has low ratio of oxygen ligands. It was synthesized in the Mn$_2$(II,II) state [6]. Even if the lower oxidation levels were stable, the number of electrons extracted (3 in total) was not enough for accommodating water splitting [6]. At least 4 electrons are required.

The goal has been to investigate if fewer than four manganese ions can be used and still accomplish water oxidation. Therefore, another complex was synthesized with higher O/N ratio than in 1 with two di-tert-butyl substituted phenolate ligands. The ligand was the trianion of 2,6-bis{[N-(2-hydroxy-3,5-di-tert-butylbenzyl)-N-(2-pyridinemethyl)amino]methyl}-4-methylphenol. It may be possible to rely on ligand adjustments to acquire more electrons.
The electron donating properties of the ligand in 2 increased and higher oxidation states could be more stabilized [7]. Oxygen which has higher electronegativity than nitrogen is a better electron donor. Although the increase of oxygen ligation would result in losing the lower oxidation states many studies have indicated that in the natural photosynthesis a higher O/N ratio is observed [7]. Electrochemical studies of 2 have proved that up to five oxidation states can be accommodated with the initial oxidation state Mn$_2$(III,III) in an 50:50 acetonitrile:water mixture [7]. Unfortunately, preliminary studies showed that neither 2 had the ability to oxidize water.

3.3.2.1 The Unsymmetric Manganese Complex
Recently a novel Unsymmetric Manganese(II,III) Complex, a dinuclear ([Mn$_2$L(µ-OAc)$_2$]ClO$_4$), where L is the X-anion of 2-(N,N-Bis(2-methylpyridyl)aminomethyl)-6-(N-(3,5-di-tert-butylbenzyl-2-hydroxy)-N-(pyridylmethyl)aminomethyl)-4-methylphenol was successfully synthesized. This complex has the similar structure as the previous complexes, but with only one di-tert-butyl substituted phenolate ligand compared to 2. The descriptive name concludes that the complex is not symmetric. It is known that the manganese ions, in PSII, are not chemically equivalent, they comprise of different ligands. Therefore an unsymmetric complex might be a better mimick of the natural system. Electrochemical studies and X-band EPR have been conducted on 3 and these results show that it could be stabilized in five oxidation states in dry acetonitrile, similar to 2. In addition it was found that these oxidation states were energetically stabilized in the presence of water [8]. This concludes that the complex is an interesting candidate for further investigations. The elucidated studies of this complex are presented in the EPR analysis (see section 6.3)
4 THE ROLE OF MANGANESE

4.1 Introduction

Since the photochemical reaction relies only on one electron transfer at each step, the production of one oxygen molecule would demand a cooperation of four oxidizing equivalents, where each equivalent undergoes a photo-chemical step, in extracting a total sum of four electrons. The manganese complex can be considered as an accumulator that stores these four oxidizing equivalents supplied by the photo-oxidized primary donor \( \text{P}_{680}^+ \). Thus oxygen evolution is said to be regulated by a “four electron gate” [3]. The electrons are transferred from water molecules to \( \text{P}_{680}^+ \) via the oxidized secondary donor \( \text{Y}_{z'} \), and further to PSI.

4.2 OEC and the S-states

![Figure 7. The Kok cycle of PSII and the time elapse for oxygen production. A schematic representation of the OEC, Oxygen Evolving Center, in the chloroplast of higher plants. From two molecules of water four electrons are stripped, one at the time, during the light-driven reactions S1→S4. In the light-independent step S4→S0, \( \text{O}_2 \) is released. \( \text{Y}_z \) represents the redox-active intermediate necessary for water oxidation [8].]

Oxygen evolution is a complex process that takes place at PSII on the luminal face of the thylakoid membrane. In order to form oxygen four electrons must be removed from two molecules of water (two electrons from one molecule of water). The redox potential for \( \text{H}_2\text{O/O}_2 \) is 0.82 V at pH 7 [3]. The thylakoid lumen has lower pH at around 5, increasing the redox potential of \( \text{H}_2\text{O/O}_2 \) up to 0.94 V [3]. Thus each of the four oxidizing equivalents (the standard reduction potential of each S states) must have an average redox potential greater than ~ 1 V. This makes the electron abstracting \( \text{P}_{680}^+ \) among the most powerful biological oxidizing agents known. (The \( \text{P}_{680}/\text{P}_{680}^+ \) couple has a potential of at least 1.12V). The charge separation of \( \text{P}_{680} \) and the dissociation of water can not be coupled in one series.
The role of the Oxygen Evolving Complex, OEC, is to overcome the problem by reducing the high energy barrier through the coupling of several intermediary oxidation steps. The Dutch scientist Bessel Kok proposed 1970 a five state cycle of “S-state” intermediates, $S_0$ through $S_4$, where the accumulation of oxidizing equivalents is described [10]. Each subscript represents the number of oxidizing equivalents abstracted from the OEC by $P_{680}$. When the S-state cycle has reached its final stage an oxygen molecule is released from the OEC.

1. Photoabsorption by $P_{680}$ followed by exitation and electron release
2. Excited $P_{680}^+$ oxidizes tyrosine residue to a neutral radical $Y_z \rightarrow Y_z^-$
3. Tyrosine radical $Y_z^-$ extracts electrons, one step at a time from the OEC, thus contributing to $S_0 \rightarrow S_4$ transitions
4. Continued photo-absorption by $P_{680}$ drives $S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, $S_3 \rightarrow S_4$ transitions

Even if the early stages of the S-state transitions have fast electron transfers, a decrease is observed as the OEC becomes more and more oxidized. Maximum oxygen evolution occurs in approximately 1 ms after the fourth photo-absorption by $P_{680}$. This is due to the transient $S_4$ state, an unstable activated complex, which quickly generates the more stable $S_0$ state, thereby resetting the cycle [10].

### 4.3 The Manganese/Calcium Ion Cluster

A model of the Mn$_4$/Ca$^{2+}$ cluster has been proposed using X-ray crystallography at 3.5 Å (Ångström) resolution [11]. In Science (2004), Ferreira et al suggest various interpretations and it is believed that “the OEC is a cubane-like Mn$_3$CaO$_4$ cluster with a mono-µ-oxo bridge to a fourth Mn ion” [11]. (The single oxygen bridge between the two manganese ions is denoted as the mono-µ-oxo bridge). In the model of the OEC, the metal-to-metal distance has been derived to 2.7 Å (in the metal-like cubane structure) and 3.4 Å for the metal-calcium distance by using Extended X-ray Absorption Fine Structure (EXAFS). The purpose of the calcium ion is not well understood but it is believed that it plays an important role in stabilizing the manganese cluster. Studies where the calcium ion is removed shows that the manganese becomes remarkably unstable by a non-complete water oxidation. Also, substitutions with other metals such as strontium ion have led to some of these conclusions. The possible mechanisms of the oxygen evolving reaction is further discussed in the paper [11].
Figure 8. The manganese cubane-like structure in the Oxygen Evolving Complex, proposed by Ferreira et. al. The image clearly shows that three manganese ions constitute a closer linkage thereby leaving the fourth manganese ion outside the cube structure [11].
5 ELECTRON PARAMAGNETIC RESONANCE - EPR

5.1 Introduction

Electron Paramagnetic Resonance, EPR (or Electron Spin Resonance, ESR) is a method that detects paramagnetic ions or molecules (with at least one unpaired electron), which absorb microwave irradiation when the sample is placed in a magnetic field. In the experiments, the microwave frequency is fixed and the magnetic field strength is varied. (Compare with NMR were the frequency is shifted and the magnetic field fixed). Absorption of microwaves occurs only when the resonance condition is fulfilled (see section 5.2.2). EPR spectroscopy can be used in biological systems to measure species with unpaired electrons such as free organic radicals and some transition metal ions. The EPR spectra of transition metal ions are usually characteristic in the number of unpaired electrons and any coordinated ligand arrangements around the metal ion [12].

5.2 EPR Theory

5.2.1 The Zeeman Effect

When an atom is placed in an external magnetic field the energy of the atom changes due to the energy of the magnetic moment in the field. This creates a splitting of the spectral lines into several sub-levels. The degeneracy of the electronic configuration is broken and lines appear with slightly higher or lower energy than the energy state in the absence of a magnetic field. The splitting of the spectral lines is called the Zeeman effect after the Dutch Physicist Pieter Zeeman who observed this phenomenon in 1896. The spacing between the splitted individual lines corresponds to the magnitude of the magnetic field, i.e. corresponding to the energy of the absorbed electromagnetic waves [13].

Since some transitions are forbidden, it does not occur between all pairs of energy levels. Therefore the selection rules for a transition induced by a magnetic-dipole process (in EPR) are [13]:

$$\Delta M_s = \pm 1 \text{ and } \Delta M_I = 0$$

where $M_s$ is the electron spin quantum number and $M_I$ is the nuclear spin quantum number.
5.2.2 The Resonance Condition

The electron possesses an intrinsic magnetic moment due to its spin. The magnetic moment has two possible orientations with two distinct energies, lower or higher energy, in the presence of an applied field. The transition states refer to the electrons spin states, clockwise \((S = +\frac{1}{2})\) or anti-clockwise \((S = -\frac{1}{2})\). When an oscillating electron magnetic radiation of the appropriate frequency \(\nu\) is applied perpendicular to the external magnetic field \(B\), transition can occur between the two spin states and the intrinsic spin is “flipped”. The difference between the two energy levels,

\[
\Delta E = E_+ - E_- = g\beta_e B \quad [1]
\]

corresponds to the energy of the absorbed photon that is required to cause a transition. Thus the resonance condition is:

\[
h\nu = g\beta_e B \quad [2]
\]

In equations [1] and [2], \(g\) represents the g-value \((g_e = 2.00232\) for a free electron), which is characteristic for each radical. \(B\) is the magnetic field strength in Gauss, \(\beta_e\) is the Bohr magneton \((9.274 \times 10^{-28} \text{ JG}^{-1})\), \(h\) \((6.6262 \times 10^{-27} \text{ Js})\) is Planck’s constant and \(\nu\) corresponds to the frequency of the absorbed photon in Hz [12].

5.2.3 The Zeeman Hamiltonian

When a free electron is placed in a magnetic field, the degeneracy of the spin energy levels is changed. This effect is called the Zeeman effect and is described in the following spin Hamiltonian equation [13].

\[
\hat{H} = g\beta_e \vec{B} \cdot \vec{S} \quad [3]
\]

\((\vec{S}\) is the vector component of the spin angular momentum operator\)

The Hamiltonian operates on \(\alpha\) \((m_s = +\frac{1}{2})\) or \(\beta\) \((m_s = -\frac{1}{2})\) spin states leading to energy splitting [13].
When a nucleus is placed in an external magnetic field it too interacts with the magnetic field creating a nuclear magnetic moment due to the protons present, each with the nuclear spin of \( I = \frac{1}{2} \). Therefore the energy levels become modified due to interactions between the nucleus and the electron causing hyperfine splitting (see section 5.2.6). This is described in the Hamiltonian equation as following [13]:

\[
\tilde{H} = \text{Zeeman interaction} + A \tilde{S} \cdot \tilde{I} - \text{Nuclear Interaction} - \text{Hyperfine Interaction}
\]

\[\alpha, m_s = +\frac{1}{2}, E_+ = \frac{1}{2} g \beta_e B\]

\[\beta, m_s = -\frac{1}{2}, E_- = -\frac{1}{2} g \beta_e B\]
5.2.3.1 Zero-Field Splitting (ZFS)
In systems with more than one unpaired electron ($S > \frac{1}{2}$), the interactions between the spins in its ground state can give rise to energy level splitting in the absence of an external magnetic field [12].

The general Hamiltonian equation, including the Zeeman interaction of the electron and the nucleus, the Hyperfine interaction and the Zero Field Splitting, is [13],
A is the hyperfine coupling constant and D is the Zero Field Constant.

5.2.4 Sensitivity - Boltzmann Distribution Law

The distribution of molecules at different energy levels depends on the energy difference between two levels. The relative distribution of molecules in the different energy states at equilibrium is described by Boltzmann distribution law [13]:

\[
\frac{N_+}{N_-} = e^{\frac{-\Delta E}{k_B T}} = e^{\frac{-g \beta_e B}{k_B T}} \quad [6]
\]

where \(N_+\) corresponds to the upper energy level and \(N_-\) corresponds to the lower energy level. For ordinary magnetic fields and temperatures the exponent in equation [6] is very small and can be approximated by \(e^{-x}(1-x)\) to [13],

\[
\frac{N_+}{N_-} \approx 1 - \frac{g \beta_e B}{k_B T}
\]

The population difference can be written as following [13],

\[
N_+ - N_- = N_- \left[ 1 - \left(1 - \frac{g \beta_e B}{k_B T} \right) \right] = \frac{Ng \beta_e B}{2k_B T} \quad [7]
\]

Equation [7] describes the increasing EPR sensitivity with decreasing temperature and increasing magnetic field. Since the microwave frequency is proportional to the magnetic field strength, increased microwave frequency corresponds to greater sensitivity for Q-band than X-band or K-band (see section 5.4) [13]. For example, when the population in the ground state is small, absorbing intense radiation can cause a more equal distribution between the energy states up to a point of saturation. Equilibrium is reached via energy transfer of the spin system in a process called spin-lattice relaxation, with the spin-relaxation time \(T_1\). Systems with long \(T_1\) (organic radicals), which are weakly coupled to the surroundings, will easily be saturated while systems with shorter \(T_1\) (organotransition metal radicals) will be more difficult to saturate [13].
5.2.5 The Electron Zeeman Factor - g-value

The g-factor is used to characterize the position of resonance and reflects the local magnetic field experienced by the electron. The electron which is a charged particle generates a magnetic field around itself. It spins both around its axis and orbits the nucleus, thus the spin magnetic dipole and the orbital magnetic dipole interacts. The g-value can be isotropic or anistropic, the latter being most common [14]. Anisotropy in $g$ arises from the coupling of the spin angular momentum with the orbital angular momentum. The spin angular momentum will always align with the applied field, but the orbital angular momentum depends on the molecular wave function. If not for the orbital contribution, the moment of the electron would be isotropic. In other words, the g-value depends on the symmetry of the system. A perfect isotropic system would have only one g-value. A fast rate of motion in all directions would average the anisotropic characters and give isotropic g-value. Since in most cases there occure a continous molecule movement, the orientation of the species changes with respect to the applied field. Thus the g-value measures an average of the electron orbital symmetry, when the molecules tumble rapidly and the local magnetic field alters frequently [12]. The g-value is defined from equation [2] as

$$g = \frac{\hbar \nu}{\beta_e B} \quad [8]$$

It can have three possible orientations $g_x$, $g_y$ and $g_z$ ($g_x = g_y = g_z$ in isotropic systems) where each independently contributes when the external magnetic field is parallel to one of the three perpendicular directions. In anisotropic systems the g-value is described by a matrix. The g-value for a free electron is always 2.0023 and the deviation depends on the applied field’s capacity to induce local electron currents [14]. The main use is to aid the identification of the species present in the sample. For transition metals the g-value can range between 1-10, whereas smaller changes are observed for organic free radicals. Since many transition metals account for large spin-orbit interactions, measurements are often conducted at low temperatures, when the spin-orbit interactions are reduced, in order to obtain resolved EPR-spectra [12].

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5.2.6 The Hyperfine Splitting Factor

Since the nucleus has an intrinsic magnetic moment, the electron can interact with it due to its own spin. This causes a phenomenon referred to as hyperfine splitting [12]. Moreover, because the magnitude of the splitting depends on the distribution of the unpaired electron near the magnetic nuclei, the spectrum can be used to map the molecular orbital occupied by the unpaired electron [15].

Consider a simple proton that has a spin \( I = \frac{1}{2} \). Then the nuclear spin quantum number has two allowed orientations, \( M_I = \pm \frac{1}{2} \). Hence there is one of two possible local fields at each position of the electron and there are then two values of the external magnetic field at which resonance occurs [15]:

\[
B = B' - aM_I + \ldots = B' \mp \frac{a}{2} + \ldots \quad [9]
\]

Where \( aM_I \) is the local magnetic field at the electron, \( B_{\text{local}} \), and \( B' = \frac{h\nu}{\beta B_0} \) is the resonant field when the hyperfine coupling constant \( a \) equals zero.

Instead of a single line, the spectrum shows two lines of half the original intensity separated by the hyperfine coupling constant \( a \), and centered on the field determined by the g-factor. When there are several magnetic nuclei present, each one contributes to the hyperfine structure [15].

There are two types of interactions [15]:

1. Isotropic interaction caused by delocalisation of an unpaired electron to the nucleus.
2. Anisotropic interaction (Dipolar Interaction) between the electron spin and the nucleus spin.

The isotropic hyperfine interaction contributes to a spin local field at the electron produced by the nuclear spin. The nucleus spin is split in \((2I + 1)\) orientations, all with different energy levels, in the presence of a magnetic field. The unpaired electron is affected by these different orientations and thus the EPR spectrum is split in \((2I + 1)\) lines, all with equal intensity. Consequently, a sample with \( n \) equivalent nuclei is then split in \((2nI + 1)\) lines. Hyperfine splitting is described as the width in Gauss [15].

Anisotropy of the nuclear hyperfine interaction arises from the dipolar interaction of the magnetic field of the electron with the magnetic field of the nucleus. In anisotropic systems the hyperfine coupling constant has three possible orientations \( A_{xx}, A_{yy} \) and \( A_{zz} \) which together build up a matrix [12].
5.3 Appearances in the EPR Spectrum

5.3.1 Intensity

In any kind of spectrum the area under the signal is proportional to the concentration of the species, unpaired spins, in the sample. The EPR spectrum is displayed as the first derivative of the absorption spectrum, as this seems to be the easiest way of interpreting the information. Such, peak-to-peak intensities are measured instead [12].

5.3.2 Linewidth

Signal broadening is affected by various reasons. These are [12]:

1. Spin-lattice relaxation
2. Spin-spin interactions
3. Electron spin exchange

5.3.2.1 Spin-lattice Relaxation

The smaller relaxation time $T_1$ the system possesses, the broader the spectral line as it is characterized by a first-order rate constant, $1/T_1$. (See equation [6]). It depends on the interactions between paramagnetic ions and thermal vibrations. $T_1$ measures the recovery rate after a perturbation when the electron experiences fluctuating magnetic fields.

5.3.2.2 Spin-spin Interactions

Paramagnetic species can induce local magnetic fields on their neighbouring molecule which alter the total magnetic field. This leads to a small shift in energy. The sum of these smaller changes contributes to a distribution in energy, resulting in broader lines.

5.3.2.3 Electron Spin Exchange

A dinuclear manganese (II,II) system, where each manganese ion possesses a spin of $I = 5/2$, have five unpaired electrons in their outer shells. These recombine such that only one unpaired electron is “alone”, thereby permitting a single EPR-signal at cryogenic temperatures. In higher temperature studies, when the spin exchange is altered, the predictions are not as easily made as additional signals appear at different magnetic fields.

Unresolved overlapping spectra may also lead to signal broadening.
5.4 EPR Instrumentation

EPR spectrometers are available in various frequency ranges since the frequency is kept constant whereas the magnetic field is variable. A list of frequencies and their corresponding microwave band is presented below.

<table>
<thead>
<tr>
<th>MICROWAVE BAND</th>
<th>FREQUENCY (GHZ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>1.1</td>
</tr>
<tr>
<td>S</td>
<td>4.0</td>
</tr>
<tr>
<td>X</td>
<td>9.5</td>
</tr>
<tr>
<td>K</td>
<td>23.0</td>
</tr>
<tr>
<td>Q</td>
<td>34.0</td>
</tr>
<tr>
<td>W</td>
<td>94.0</td>
</tr>
</tbody>
</table>

X-band EPR is widely used since the sample volume is convenient (tube diameter ~ 4 mm), giving enough information from the obtained spectra. Though, for numerous reasons, people tend to use other microwave bands that operate at either higher or lower frequencies. Q-band offers higher sensitivity for small samples and a considerable improvement in g-factor resolution compared to X-band. The cylindrical Q-band resonator of the design, ER 5106QT/W, operates at 34 GHz in which the resonator frequency is slightly tunable taking in account some frequency changes due to sample insertion and temperature variations (see section 6.2.1). In addition to the cavity there exists a sample ruler for reproducible positioning of point samples. The resonator is fitted to a helium cryostat of ER 4118CF as well as quartz glass in which the sample is stored. Normal glass tubes are not used simply because they can contain impurities such as metal ions that can disturb the measurements and contribute to background signals.

<table>
<thead>
<tr>
<th>Resonance Frequency</th>
<th>34 GHz$^{(1)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max. Modulation Amplitude</td>
<td>20 G</td>
</tr>
<tr>
<td>Range of Modulation Frequencies</td>
<td>100 kHz</td>
</tr>
<tr>
<td>Typical Modulation Limit</td>
<td>15 %</td>
</tr>
<tr>
<td>Sample Temperature Range</td>
<td>3.8 – 323 K</td>
</tr>
<tr>
<td>Weak Pitch Signal-To-Noise Ratio</td>
<td>2000:1</td>
</tr>
</tbody>
</table>

Figure 11. A list of the different operating bands that are available on the market and their corresponding microwave frequencies [16].

Figure 12. Different operating parameters for the Q-band cavity, ER 5106QT/W. (1) adjustable by cavity length [16].
6 EXPERIMENTAL STUDIES

6.1 Introduction and Problem Statement

EPR has for a long time been used as a fingerprint for characterizing manganese complexes in different valence states. As discussed before, the splitting pattern \((2nI + 1)\) depends intrinsically on the nuclear spin. For instance, ordinary mononuclear Mn(II) ion with nuclear spin \(I = 5/2\) shows six lines in the EPR hyperfine splitting spectrum (figure 14). Coupled manganese dimers in different valence states result in more complex and complicated spectra with overlapping combinations. Therefore a straightforward analysis is in general not possible, whereas a combination of several application methods is required. Nevertheless altering frequency strengths in the experiments alone can contribute to an evaluation of several EPR parameters, thereby creating increased resolution in the obtained data.

Generally, the mixed valence \(\text{Mn}_2(\text{II,III})\) ground state \((S = 1/2)\) shows strong EPR signal at cryogenic temperatures (extremely low temperatures) in \(g\sim2\) region resolving 17-20 hyperfine lines. Temperature raise of one or two Kelvin give rise to excited state transitions \((S = 3/2\) and \(S = 5/2)\), observed in the low magnetic field regions [17]. Since the electron spin exchange is more extensive at higher temperatures it is difficult to predict the number of lines in the spectrum. Therefore the experiments are conducted at very low temperatures.

Unsymmetric \(\text{Mn}_2(\text{II,III})\) Complex has previously been characterized by X-EPR (9.5GHz) [17]. Though it shows similar features as the symmetric manganese complex in the obtained EPR-spectra, i.e. many overlapping positions with the symmetric \(\text{Mn}_2(\text{bpmp})(\mu-\text{OAC})_2\) X-EPR spectrum. Thus Q-band EPR (34 GHz) experiments, with higher magnetic field strength, could lead to more information of the unsymmetric complex. As additional parameters, the \(g\)-value and the hyperfine coupling constant, can help in EPR simulations, further interpretations and suggestions for a comparative model can be proposed based on the magnetic interactions. Most importantly understanding the arrangement and behavior of the different manganese complexes can yield further insights of their intrinsic properties.

Even if problems were encountered with Q-band technique, e.g. buried signals under the cavity noise at higher magnetic field strengths, it offers higher sensitivity, increased resolution and less broad signals than X-band. These goals alone will give rise to more parameters useful for simulation techniques and distinguish the unsymmetric manganese complex from the symmetric.
6.2 Experimental Design

6.2.1 The Experimental Set-Up

In order to manage a successful experiment each individual sub-system must function properly. A cold temperature EPR experiment requires much preparation in the instrumental setup. This is discussed in detail in section 6.2.2.2. A brief explanation of the crucial instruments that are necessary and their individual contribution in acquiring reasonable spectra follows.

The experimental set-up of the EPR spectrometer used is outlined in the figure below. (Detailed picture of the cavity and cryostat is found in Appendix F and G).

Figure 13. The Electron Paramagnetic Resonance Set-Up (Artificial Photosynthesis Research Lab, Uppsala University). See Appendix D for magnification of the cavity. Photo by Ji-Hu Su.
Generally an EPR spectrometer is composed of several key factors:

1. Microwave Bridge
2. Cavity
3. Electromagnets
4. Signal Channel (“Console”)
5. User interface (Computer)

6.2.1.1 The Microwave Bridge
The microwave bridge functions both as a microwave source and a detector of the transmitted waves from the cavity. The microwaves are generated through a gun-diode semiconductor oscillator which is widely used to produce lower power ratio signals at microwave frequencies. They can operate from a few GHz up to 100 GHz. The signal microwave frequency depends on the thickness of the active region. Since this is often linked to a wave guide, the frequency is not permanent and manual adjustments can be done during the tuning process before each experiment. This is made by placing an adjustable screw into the waveguide cavity [16].

The microwave radiation from the cavity is reflected to the detector, which consists of a diode that converts the microwave power to electric current, via a circulator that acts as traffic circle by changing the route of the microwaves. The reflected microwaves are directed only to the detector and not to the source. Detection is further improved by modulating the signal. If the modulation is less than the absorption signal linewidth, the detector is sensitive to the phase of the signal, then the derivative of the absorption signal appears. To control the power output of the microwave source there exists an variable attenuator that blocks the flow of microwave radiation [16]. Thus one can more accurately determine and control the amount of microwave power exposed to the sample.

6.2.1.2 The Cavity
The sample is held in a microwave cavity which consists of a metal box that aids the amplification of weak signals from the sample. The cavity is itself positioned into a cryostat that remains vacuum in the system. The sample which is surrounded by two large magnetic coils is swept with a magnetic field through the resonance condition. The shape of the cavity is designed so that resonance at microwave absorption will be optimized [16]. Thus the dimensions of the cavity size are different for different frequencies.

The quality factor of the cavity, Q-value, describes how well the cavity stores the microwave energy [14].

\[ Q = \frac{2\pi \text{ (energy stored)}}{\text{energy dissipated per cycle}} \]

The energy dissipation per cycle represents the amount of energy loss in one microwave period. The higher the energy loss, i.e. Q decreases, the lower the sensitivity of the
spectrometer. Some of the microwave energy can be lost to the side walls of the cavity since electric currents are generated, which contribute to heat production. In order to reduce the origin of electric currents inside the cavity, the sample is placed in an electric field minimum and a magnetic field maximum. Thus bigger signals and higher sensitivities can be obtained.

The amount of microwave radiation into the cavity and the transmission back to the detector is determined by the iris [16]. This is a hole that operates by carefully controlling the impedans (resistance) of the cavity and the waveguide. There exists a screw in front of the cavity where manual adjustments of the iris can be made.

6.2.1.3 The Signal Channel
The signal channel is built into the console which contains the required electronics for phase sensitive detection. Phase sensitive detection with magnetic field modulation increases the system sensitivity. The magnetic field is continously modulated sinusoidally at the modulation frequency. When an EPR signal occurs, the field modulation sweeps threw the signal and the transmitted microwaves from the cavity becomes amplitude modulated at the same frequency. A reference signal, which is sensitive to signals with the same modulation frequency and phase as the field modulation, is compared to the modulated signal. The signals which do not fullfill these requirements are suppressed. Even if higher modulation amplitudes give rise to increased intensities, too high modulation amplitudes can subject broadening and distortion of the signal [16]. A good rule of thumb is to use a modulation amplitude that is less than the linewidth of the signal. Otherwise, there may occur difficulties in resolving signals from eachother.

6.2.1.4 The Magnetic Field Controller
Whithout a magnetic field controller it would be difficult to control the magnetic field sweep through the sample. The controller sets the field values, the sweep time and the current that flows in each magnet coil (windings). The controller regulates the magnetic field by receiving a reference value, in numerous times, which correspond to the magnetic field value. In between the two magnets a Hall probe is placed. This is influenced by the magnetic field such that it produces a voltage which is dependant of the field. The controller can then regulate the field by comparing the voltage produced by the Hall probe with the previously set reference voltage. Whenever there is a difference in voltage the magnetic power supply changes the amount of current that flows through the magnets and hence the magnetic field [16].

6.2.1.5 The User Interface
Bruker supplies an appropriate application package called WIN-EPR Aquisition Software (based on Windows '95) [16]. Configuration can be made for various types of measurements, i.e. different resonators.
6.2.2 Experimental Operation

6.2.2.1 Sample Preparation
Powder \([\text{Mn}_{2}(\text{II,III})\text{L}(\mu-\text{OAc})_2]\text{ClO}_4\) (synthesized at Stockholm University), with the approximate molecular mass 1000 g/mol (including the corresponding anion of the complex, \(\text{ClO}_4\)) was solved in solvents with different dielectric constants. The study was initiated with dimethyl sulfoxide, DMSO. This solvent seemed to have relatively high dielectricity, which contributed difficulties in tuning the cavity, resulting in an unstable system. Therefore the study was followed by a combination of solvents with the ratio 2:1, dimethyl formamide:acetonitrile solution. The final concentration level was 0.5 mM. Care was taken when the sample solution was poured in Q-band quartz tubes since air bubbles might be produced which can disturb the cold temperature measurements. Afterwards, the quartz tubes are kept frozen in liquid nitrogen at 77K.

6.2.2.2 Q-band EPR Preparation
The cryostat is placed between the two magnet coils on a supporting plate with the cavity placed within the cryostat (see figure 13). The cavity is attached to a microwave guide connecting it to the microwave bridge. The cryostat is isolated by a vacuum between the sample compartment and the surroundings. The vacuum is pumped to \(\sim 10^{-6}\) bar for approximately an hour. Low pressure nitrogen gas is flushed through the cryostat for some minutes. Before the sample quartz tube is inserted into the cavity, it is fixed to a sample rod of corresponding size such that the sample protrudes approximately 3 cm. It is crucial that the tube is held tightly in the sample rod to prevent vibrations which can lead to increased noise levels during measurements.

Cool-down process starts by the insertion of a helium transfer line, which is earlier purged with low pressure inert helium gas, into the cryostat. The transfer line is connected to a helium tank which with the aid of a pump controls the flow of liquid helium circulating the system. After a while when the cavity is bathing in pure liquid helium, the temperature stabilizes around 4K (the boiling point of liquid helium). Cavity tuning is made manually in several steps through cavity frequency and iris coupling adjustments. This is probably the most important step in acquiring quality spectra. Necessary parameters operating at Q-band are set to the computer software program prior to measurements. The most important parameter that needs to be adjusted is the magnetic field range. For example, a \(g\sim 2\) signal in X-band appears at lower magnetic fields than in Q-band.

6.2.2.3 Execution of Experiment
Spectra are recorded and collected in multiple scans. Addition of several scans reduces the noise level and insures reproducibility of the signal. The more the scans the better the signal to noise. Desired temperature alterations are adjusted by the amount of helium flow that reaches the system. i.e. the valve of the transfer line is either open or closed for temperature decrease or increase respectively.
6.3 Analysis and Discussion

6.3.1 Q-band EPR of Mn(II)

In order to assure sustainable results, ordinary Mn(II)Cl₂ in buhan-1-ol (final concentration level 0.5mM) was measured in Q-band at room temperature. Mn(II) is known to have a 6 line signal at g~2, which many times have been proved in X-band. The acquired Q-band spectrum below proves the 6 line character.

![Figure 14. Q-band EPR spectrum displaying mononuclear Mn(II) in buthanol solution (0.5mM). The signal spans between 11 800 and 12 500 (g = 2.0076) Gauss with the peak split of approximately 100 Gauss. Spectrometer settings: Frequency, 34.18 GHz; Modulation frequency, 100 kHz; Modulation amplitude, 10 Gauss; Microwave power, 6mW.](image)

6.3.2 Q-band EPR of Unsymmetric Mn₂(II,III) Complex

The experimental study continued with Q-band EPR of the Unsymmetric Mn₂(II,III) Complex, which was the purpose of this thesis. Obtained Q-band spectra at 5K (see figure 15, Appendix A and Appendix B) show that the signal is much more difficult to distinguish (compare with previous Mn(II) spectrum) due to the ambiguity. In figure 15 (including Appendix A and B) it is concluded that the signal ranges from 11 400 to 14 000 Gauss. Depending the peak resolution, which is buried in some noise, it is estimated that the signal contains 14-17 lines in the g~2 region, similar to the previously conducted X-band study of the complex (see Appendix C).
The peak split (peak-to-peak distance) between hyperfine lines in figure 15, is approximately 120 Gauss (varying in different regions of the spectrum). This is in well agreement with the preliminary X-band studies of the unsymmetric complex where the separation between hyperfine lines is 125 Gauss (see Appendix C). If to investigate the hyperfines for the unsymmetric complex and the symmetric in Q-band, it is clear that they are more resolved for the unsymmetric complex. Compare with the published spectrum of Mn$_2$(bpmp)(µ-OAC)$_2$ in Appendix D.

Excited state transitions at g~4 and g~6 for the unsymmetric complex are expected but could not be asserted due to baseline problems.

Figure 15. Q-band EPR of the Unsymmetric Mangnaese(II,III) Complex. Spectrometer settings: Frequency, 34.18 GHz; Modulation frequency, 100 kHz; Modulation amplitude, 10 Gauss; Microwave power 1.9 mW; Temperature, 5K. See additional spectra in Appendix A and B.
6.3.3 EPR Simulations of Unsymmetric Mn$_2$(II,III) Complex

By simulating the X-band spectrum of Unsymmetric Mn$_2$(II,III) (not yet published), it was possible to calculate the hyperfine coupling constants of the isolated manganese ions. The constants were $A_{\text{Mn(II)}} = 83 \cdot 10^{-4} \text{ cm}^{-1}$ and $A_{\text{Mn(III)}} = 65 \cdot 10^{-4} \text{ cm}^{-1}$. The large difference between these values indicates that the unpaired electron is localized on one Mn-ion. A first attempt of simulating the Q-band EPR spectrum of Unsymmetric Mn$_2$(II,III) Complex has also been made. Though this does not agree in large extent with the observed spectrum, it is yet to be investigated. Further studies might yield better simulation parameters in order to perform calculations that enable reliable predictions of the molecular structure around the manganese ions.
7 CONCLUSIONS

The master’s thesis has been emphasizing on the ability to produce Q-band EPR spectra of the Unsymmetric Mn$_2$(II,III) Complex. Obtaining a reduced noise spectrum of the complex resulted in a difficult task, considering the extreme sensitivity and the practical challenge in handling a Q-band instrument.

Data results shows that the unsymmetric Mn$_2$(II,III) complex has a signal at g~2, ranging from 11 400 to 14 000 Gauss. The spectrum displays distinct hyperfine lines of 120 Gauss.

It is concluded that for the unsymmetric signal the number of lines and the distance between hyperfines are similar in X-band as in Q-band. This indicates that the g-value is not as anisotropic after all. Either there only exists one single g-value for the mixed valence system or the three different g-values (g$_x$, g$_y$, g$_z$) are lying in close proximity. This is not distinguishable in the conducted Q-band EPR measurements. It would require higher frequency studies (W-band EPR, 94 GHz) in order to rule out g-value anisotropy.

As previously discussed, unsymmetric Mn$_2$(II,III) and Mn$_2$(bpmp)(µ-OAC)$_2$ possess similar features in the X-band spectrum but differ in the Q-band spectrum. Comparisons with published material ([18]) of Mn$_2$(bpmp)(µ-OAC)$_2$ in Q-band and X-band (Appendix D and E) provide that information, e.g. Mn$_2$(bpmp)(µ-OAC)$_2$ displays weaker hyperfines in Q-band.

Reliable X-EPR simulation parameters show the chemical feature of the mixed valence state manganese ion system. The single unpaired electron seems to be localized to one Mn-ion due to the large anisotropic coupling constant obtained. Further conclusions in molecular properties are not eligible at this moment since e.g. a proper Q-EPR simulation spectrum is not acquired, but the characterization study is definitely interesting to pursue.

Without a doubt, the conducted Q-band EPR measurements of the unsymmetric Mn$_2$(II,III) complex have contributed to promising information in creating a future artificial system. Further studies of the Unsymmetric Mn$_2$(II,III) Complex are necessary and yet to be expected.
8 EVALUATION

8.1 Optimizing Sensitivity

In order to obtain spectra of high quality, instrumental sensitivity is crucial. Below are some guidelines and suggestions in how to optimize different settings; minimization of signal to noise ratios, decreasing usage time and acquiring good data.

8.1.1 Parameter Selection

- **Receiver Gain**
  In order to obtain good signal to noise ratio it is important to have stable receiver gain or else the signal channel can get overloaded.

- **Modulation Amplitude**
  It is a good reference if the modulation amplitude is approximately the width of the EPR signal. Too high modulation amplitude can contribute to broadening of lines.

- **Microwave Power**
  The power required for individual experiments will be dependent upon factors as concentration, solvent and temperature. Therefore to determine the optimal power, several microwave power settings must be attempted.

- **Time Constant**
  Too fast time constant contributes to unresolved signals with excessive noise. Even if the time constant filters out the noise, too slow time constant contributes to a diminished and distorted signal.

8.1.2 Instrumental Factors

- **Noise Generated by Sample**
  If the sample is not held properly in the sample rod, vibrations may occur that can contribute to noise inside the cavity. Also, gas bubbles can be produced and contribute to noise when the solution is poured into the sample tube or when the sample is freezed in liquid nitrogen. The noise can for example make an impact on the sensitivity of the cavity and the frequency leading to noise spikes on obtained spectra.

- **Controlled Environment in the Surroundings**
  It is important that the temperature is not fluctuating too much and that humidity is remained at a low level. Otherwise problems with temperature stability and water condensation can arise when cooling the sample. Condensation can be diminished by purging with inert gas such as helium or nitrogen prior to cool-down.
Sample Positioning in the Cavity
This is most important for samples with solvents that have high dielectric constants. Improper sample positioning may contribute to altered microwave field mode pattern in the cavity, resulting in reduced sensitivity.

Microwave Guide
Tight connection between the microwave bridge and the cavity ensures maximum power transfer to the sample. Therefore, the ends of the microwave guide should be tightly sealed to the bridge and the cavity.

Cavity Tuning
The cavity iris and the cavity length are extremely sensistive. When tuning the cavity care must be taken such that adjustments are not run as far as into the end positions.
9 FUTURE WORK

9.1 The Small Scale

The results show that the amount of lines for the unsymmetric Mn$_2$(II,III) (~16 lines) is similar in Q-band as in X-band. Then, does this conclude that the Q-band spectrum have one consistent g-value or rather numerous g-values that are not in visible range of the measured spectrum? To investigate this fact, studies at even higher frequencies than Q-band, i.e. W-band, would be necessary. This instrument is unfortunately not available at the laboratory in Uppsala.

Water binding by the unsymmetric complex can be studied in a solution with different mixtures of D$_2$O and followed by EPR in addition with FTIR for complete interpretations. This is currently under investigation with Mn$_2$(bpmp)(µ-OAC)$_2$.

A further application would include the unsymmetric manganese complex in a supercomplex which upon stepwise light induced oxidation enables intramolecular electron transfer. Unsymmetric Mn$_2$(II,III) would be linked to photosensitizer ruthenium molecule and redox-active cobalt ion as electron acceptor. The study could help in providing information on the efficiency of electron transfer, the amount of electrons available for extraction from the manganese complex and the stability of higher manganese valence states.

9.2 The Big Scale

Other propositions of super-complexes, than the earlier mentioned cobalt ion or NDI molecule as final electron acceptors, are also suggested and they are currently under investigation on sub-system levels. The Ru(bpy)$_3$ can be linked to iron-sulfur center via a quinone intermediate. In nature, algae, which are members of the hydrogenase family (contains iron-sulfur centers), are capable of producing hydrogen gas from hydrogen ions through enzymatic activity. In the future, linkage of Ru(bpy)$_3$, quinone, Fe-S center and manganese complex can lead to a super system where electrons are extracted from water on the manganese side and hydrogen is reduced on the Fe-S side. (Electron transfer from the manganese complex to Ru(bpy)$_3$, quinone and Fe-S center) [5].

Figure 16. The natural hydrogenase enzyme to the left and an artificial bio-mimick to the right. The catalytic center consists of Fe and S similar to the hydrogenases. Upon light-exitation of ruthenium (electrons transferred to Fe-S center), the Fe-S center reduces hydrogen ions to hydrogen [5].
9.3 Outlook

It is not yet fully understood how to create the best super-complex necessary for oxidizing water and producing hydrogen gas, but we are today aware of how to convert sunlight into chemical energy and chemically synthesize energy rich fuels. It is left to see how this connection will appear in the future.

Though a working super-complex system is to be expected in a couple of years, the actual solar energy apparatus might take up to 15 to 20 years. There are still many aspects to fulfill but collaborations from scientist worldwide appear promising and the goal of fuel production from water and sunlight might not be so unrealistic to achieve after all.
BIBLIOGRAPHY


APPENDIX A

Fig 17. Q-band EPR of Unsymmetric Mn_{II,III} in dimethyl formamide:acetonitrile (2:1) (final concentration 0.5mM) at 5K. The signal is displayed at g~2 and is approximately 2800 Gauss broad with hyperfines of 120 Gauss. (The hyperfine lines are more evident in figure 15). Spectrometer settings: Frequency, 34.23 GHz; Modulation frequency, 100 kHz; Modulation amplitude, 10 Gauss; Microwave power, 1.9 mW.
Figure 18. Q-band EPR of Unsymmetric Mn$_2$(II,III) in dimethyl formamide:acetonitrile (2:1) (final concentration 0.5mM) at 5K. The signal shows similar patterns as in previous figures 15 and 20, though the hyperfines are more resolved (see in the range of 12 400-12 800 Gauss) here. Spectrometer settings: Frequency, 34.18 GHz; Modulation frequency, 100 kHz; Modulation amplitude, 10 Gauss; Microwave power, 1.9 mW.
Figure 19. X-band spectrum of Unsymmetric Mn$_2$(II,III) at 6K. At g~2 the signal consist of 16 lines between 2300 and 4500 Gauss and has a peak split of 110-140 Gauss. Spectrometer settings: Frequency, 9.4 Hz; Modulation frequency, 100 kHz; Modulation amplitude, 15 G; Microwave power, 16 mW.
Figure 20. Q-band spectrum of \([\text{Mn} \text{bpmp}(\mu\text{-OAC})_2] \text{(ClO}_4)_2 \cdot \text{H}_2\text{O}\) in acetone:toluene:ethanol (2:1:2) solution at 20 K [18].
APPENDIX E

Figure 21. X-band EPR spectrum of \([\text{Mn}_2(\text{bpmp})(\mu-\text{OAC})_2] \cdot (\text{ClO}_4)_2 \cdot \text{H}_2\text{O}\) in acetone-toluene:ethanol (2:1:2) at 7.5 K. (A) represents the observed spectrum and (B) the simulated spectrum [18].
Figure 22. The cavity is surrounded by electromagnets. Liquid helium is flushed into cryostat through the helium inflow inlet. See section 6.2 for further information. Photo by Ji-Hu Su.
Figure 23. Magnification of cavity. The microwave guide connects the cavity on one end and the microwave bridge on the other, enabling transportation of microwaves to the sample and back to the detector. The sample rod with the sample quartz tube protruding (not shown on figure), is placed such that it is completely penetrating the cavity. Photo by Jordanos Kiflemariam.