Connecting dots between natural and artificial photosynthesis : magnetic resonance studies on light harvesting and the water oxidation reaction centre

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CHAPTER 1

General Introduction

1.1. Photosynthesis

After 3 billion years of evolution, nature has provided us with a wonderful machinery to convert sunlight into storable chemical energy: photosynthesis[1,2]. In the process of photosynthesis the waste product is oxygen, which sustains the life of oxygen-consuming organisms. Photosynthesis takes place in many different organisms. In higher plants and green algae, photosynthesis occurs in thylakoid membranes, which are present in chloroplasts. The stacked membranes are called grana whereas non-stacked membranes are known as stromal thylakoids[3].

Photosynthesis involves three consecutive steps. In the first step light energy is absorbed by light harvesting antenna complexes and funneled to a reaction center[4,5]. In the second step, the absorbed light energy is converted and stored into chemical forms as NADPH and ATP[6,7]. The third step involves using NADPH and ATP to assimilate the carbon or carbon fixation. In this final step the energy is stored in sugars, which are a form of cellular biochemical energy[8].

In higher plants, the Photosystem II (PSII) is selectively located in grana thylakoid membranes. It is practically possible to separate and isolate thylakoid membranes that contain mostly PSII by using a detergent-based method[9,10]. The PSII particles are capable of producing oxygen on illumination in the presence of an external artificial electron acceptor. Such PSII particles are used in our experiments in chapter 2.

1.2. Photosystem II and Water Oxidation Complex

Photosystem II performs water splitting, oxygen release and protonation of plastoquinone to store the electrons extracted from water in a chemical redox carrier. The detailed structure of Photosystem II has been studied and resolved by X-ray crystallography and it has been estimated that the core of the Photosystem II complex contains about 35 Chl molecules, 2 Pheophytins, 11 β-carotenes and more than 20 lipids[11–13]. It has been estimated that each Photosystem II converts the energy collected by approximately 200-300 Chl molecules[14–17].

The luminal side of Photosystem II contains the water splitting machine, the oxygen evolving complex. The structure of the OEC has recently been resolved to a resolution of 1.9 Å by X-ray crystallography by Umena *et al.*,[13]. The catalytic site of the OEC contains 4 Mn and a Ca^{2+} ion and Cl^- ions, which are required for stabilization of the structure and for proper functioning of the OEC[18–21].

1.3. Light Harvesting Complex II

All oxygen evolving photosynthesizes, like higher plants and green algae, contain similar organization of their photosynthetic apparatus which appears to be highly conserved across species and taxonomic boundaries during evolution. This functional unit should represent an effective and robust machinery that adheres to a restricted set of key engineering principles to adopt to different growing conditions on Earth and to environmental stresses[22–24]. Above all, the photosynthetic apparatus must have flexibility with respect to continuously changing radiation conditions during the daily solar cycle and yearly seasonal cycle. Last but not least, short term variations due to different shading conditions must be balanced, for example in light spots on the ground. Understanding the underlying mechanism and high flexibility of light adaptation by the peripheral antenna is a major challenge in photosynthesis research.

The LHC II complex is trimeric and each monomer contains 8 chlorophyll *a*, 6 chlorophyll *b*, 2 luteins, 1 neoxanthin and 1 violaxanthin and the structure of LCH II of pea is shown in Fig.1. LHC II complexes are involved in regulatory mechanisms to avoid photodamage from incoming light energy[5,25–28]. Under high sunlight conditions, the LHC II antenna can rapidly change from the light harvesting state to a photoprotective state. The excess energy is dissipated as heat, by mechanism called non-photochemical quenching (NPQ)[29–32]. From the LHC II major light harvesting complexes the excitation energy is transferred via the core light-harvesting complexes to the reaction center, where water is oxidized by the Mn cluster of the oxygen evolving complex.

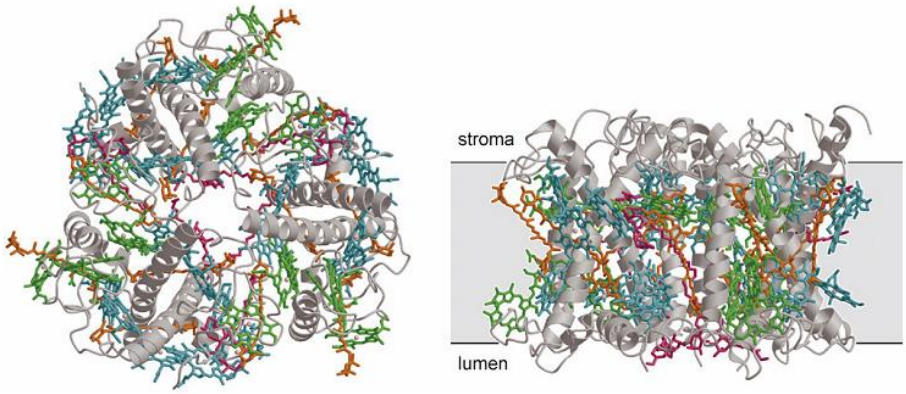

Figure 1. Structure of LHCII of pea[27]. Top view (left panel) and side view through the membrane (right panel)

1.4. Rapid Freeze Quenching

Rapid Freeze Quenching is one of the few methods that are used to study the catalytic mechanisms of enzymes through the analysis of transient intermediates[33–37]. Rapid freeze-quenching was developed by Graham, Ballou and Palmer in the nineteen sixties and seventies[38,39]. Initially the method was developed for studying the redox-enzyme kinetics with EPR spectroscopy, since the continuous and stopped flow methods were not suitable for EPR spectroscopy.

The rapid freeze-quenching set-up is basically a continuous flow instrument. The flow is generated by a drive ram present in HPLC pumps, pushing two syringes, one loaded with enzyme and the other one contains the substrate activate the enzyme reaction. After mixing the sample is delivered through a nozzle to a cryo-bath, where the reaction is rapidly quenched. The sample aging time is varied by changing the length of the nozzle tubing. To quench the reaction either cold isopentane or liquid ethane or liquid nitrogen were used.

The dead-time or minimum total sample aging time τ_a can be formulated as follows,

$$\tau_a = \tau_m + \tau_t + \tau_q$$

In which τ_a is the total aging time of the sample, τ_m is the mixing time, τ_t is the transport time and τ_q is the quenching or freezing time. Rapid freeze-quenching methods generally

involved 40 μs mixing time, 1 to 2 ms sample delivery time and 4 to 6 ms for the quenching time, yielding a total dead time of 5 to 7 ms[40–43].

By improving the methods used in rapid freeze-quenching the total dead-time was significantly improved to 130 μs by Cherepanov and de Vries[44]. This was achieved by using a stainless steel mixer base with micro-channels of 50 μm. The mixing time was determined at less than 2 μs and the cold isopentane is used as quenching medium.

The RFQ method developed by Cherepanov and Simon de Vries is used in this thesis to study the enzyme mechanisms. The two channels in the mixer were altered to one channel and the Photosystem II sample was illuminated with a high power red laser on the flow path just before quenching. The Photosystem II is quenched most effectively with cold isopentane or liquid nitrogen.

1.5. Solid State NMR

NMR chemical shifts depend on not only the different type of nucleus but also the orientation of nucleus to the static magnetic field B_o. In liquid state, the molecules exhibit Brownian motion and tumble rapidly in the order of nanoseconds to picoseconds. So the orientation-dependent chemical shift contributions such as chemical shift anisotropy and dipolar interactions are not present. In the solid state, the molecules are rigid and dipolar interactions are present, resulting in a powder like pattern. The NMR signal consists of contributions from molecules in different orientations. This is an important difference between two commonly-used NMR spectroscopies; liquid and solid state NMR.

The powder pattern spectra of Solid State NMR contains a wealth of information, but it lacks site specific information, which are used for characterization of molecules and structure determination of biological samples. To obtain high resolution Solid State NMR spectra we need to remove the anisotropic interactions. This can be achieved by mechanical rotation at high speed at an angle of 54.74^o also known as magic angle spinning as shown in fig. 2. The high speed rotation of the sample, which is packed in a rotor oriented at magic angle with respect to the static magnetic field B_o, results in disappearance of the anisotropic part and removes the anisotropic line broadening resulting in narrow lines[45,46].

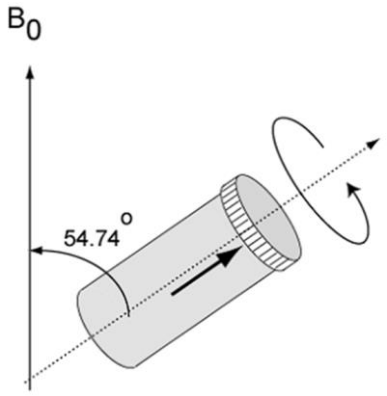

Figure 2. Depiction of the MAS technique. The sample is filled in the rotor and rotated at an anlge of 54.74° (magic angle) with respect to magnetic field B_0.

1.5.1. Cross-Polarization

Cross-polarization method is another important methodology in solid state NMR. There are two types of NMR active nuclei; one type are abundant spin nuclei like ^{1}H, ^{19}F and the other type are dilute spin nuclei like ^{13}C and ^{15}N. The former nuclei are highly natural abundant and the later ones have low natural abundance. When dealing with dilute spin nuclei in Solid State NMR, generally the signal is weak, due to long spin-relaxation time T_1. So, large number of scans and averaging of the signals is required in direct polarization experiments for adequate resolution and good signal to noise ratio.

To resolve the problem for dilute spin nuclei in Solid State NMR, cross-polarization methods are used. Cross polarization works by transferring the magnetization from abundant spin nuclei to dilute spin nuclei via their heteronuclear coupling interactions. Cross polarization enhances the magnetization of dilute spin nuclei and increases the signal to noise ratio. So less number of scans is required to get a good signal. Additionally the recycle delay, the time between the scans is reduced. The recycle delay depends on the system to return to equilibrium with B_o, which is governed by the abundant spin nucleus spin-lattice relaxation time[47,48].

Now consider the ^{1}H-^{13}C spin pair as an example and they are in a double rotating frame, which means that the magnetization of ^{13}C and ^{1}H precess about Bo. The cross polarization process starts with preparation of transverse magnetization of ^{1}H and it is maintained along the rotating frame x or y axis by using the contact pulse in a given period of time. Simultaneously another contact pulse is given to ^{13}C in order to create

transverse magnetization along its x or y rotating frame axis. The condition for transferring magnetization from ^{1}H to ^{13}C, the rotating frame energy level separation for the given two nuclei must be same. This is known as Hartmann-Hann matching condition[49]:

$$(\gamma_{1H})\boldsymbol{B}_1^{1H} = (\gamma_{13C})\boldsymbol{B}_1^{13C}$$

1.5.2. Heteronuclear Correlation experiment

Solid-state cross polarization magic-angle-spinning (CP-MAS) Frequency Switched Lee Goldburg (FSLG) Heteronuclear Correlation (HETCOR) spectra were obtained in a magnetic field of 750 MHz with the pulse sequence as shown below(see Fig.3). This experiment correlates the high-resolution proton spin signals with carbon spin signals. The correlation is obtained when ^{1}H and ^{13}C nuclei are dipolar coupled, it is therefore a through space correlation. The pulse sequence starts with preparation of a 90^{o} pulse. Subsequently frequency switched Lee-Goldburg (LG) pulses were used to remove the large homonuclear dipolar couplings[50,51]. Mixing was achieved by the cross polarization pulse during contact time. During this period the magnetization transfers from proton to carbon. During the carbon acquisition the protons are decoupled from carbon by using TPPM decoupling scheme.

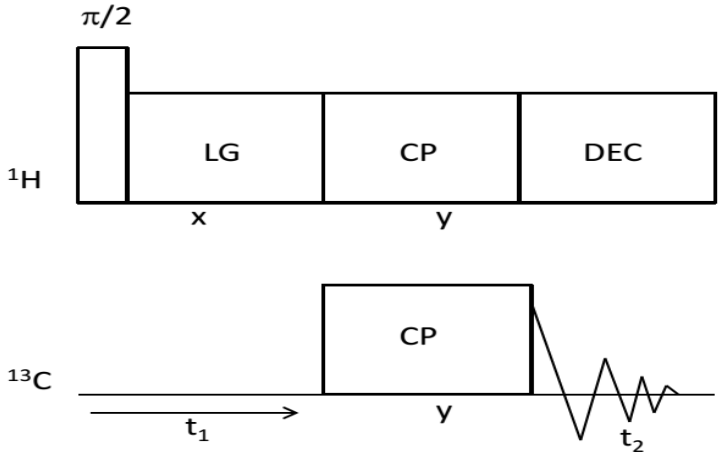

Figure 3. Hetcor ^{1}H-^{13}C LG-CP pulse sequence; this pulse sequence starts with 90^{o} preparation pulse followed by the *Lee-Goldburg* decoupling during t_1. After that cross-polarization pulses are applied in mixing time on both ^{1}H and ^{13}C. During t_2 ^{13}C FID is observed.

1.6. Electron Paramagnetic Resonance

Electron paramagnetic resonance is called as electron spin resonance. It is a spectroscopic method based on observation of resonance absorption of microwave power by unpaired electron spins in an external magnetic field. When an external magnetic field B applied, a lower energy level is formed in which the electrons are aligned with external magnetic field, and a higher energy level is formed in which the electrons are aligned in the opposite direction of the magnetic field. The energy level difference is given by

$$\Delta E = g\beta B = h\nu$$

By varying the external magnetic field B, the difference in energy levels also changes. Resonance condition emerges when the energy of the microwaves is equal to the difference in energy levels. By observing the EPR spectra, three important parameters can be obtained: the g-factor, the width of the absorbed line and nuclear-hyperfine interactions that give rise to extra lines.

This thesis focus on three projects related to natural and artificial photosynthesis. Major light harvesting complex antenna photo protection mechanism, construction and working of novel flash excitation and rapid freeze quench instrument and structural determination of artificial light antenna complexes by using MAS NMR are the scope of this thesis.

Chapter 2 of this thesis describes the construction of an instrument which combined flash excitation and rapid freeze quenching to study the structural changes during water oxidation mechanism of Photosystem II . The construction involves novel methods in connecting the dark and light parts of flash methodology are explored.

In chapter 3 the role of Arg-Glu ion pair is investigated in conformational switch from light harvesting to photo protection mode in high light conditions of major Light Harvesting Complex II with MAS NMR and selective labeling of Arg.

MAS NMR studies alone can give the self-assembled structure of Zinc amino chlorines was investigated in the chapter 4. Finally chapter 5 discuss about the outlook and future experiments.

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