Ultrafast transient absorption spectroscopy : 1) Dynamics of carbonyl stretching vibration in metal carbonyl. 2) Applications to photosynthetic systems

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**Ultrafast Transient Absorption Spectroscopy: 1)
Dynamics of Carbonyl Stretching Vibration in Metal
Carbonyl. 2) Applications to Photosynthetic Systems**

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Abstract

This thesis is focused on the setup and application of ultrafast transient absorption spectroscopy. Ultrafast transient absorption spectroscopy is a four-wave-mixing technique which is also known as ultrafast pump probe spectroscopy. The information on the mechanistic and kinetic details of molecular systems can be provided through this technique.

In the first part of the thesis (Chapter 2), the dynamics of carbonyl stretching vibration in $\text{Mn}_2(\text{CO})_{10}$ was studied by ultrafast infrared pump probe spectroscopy. Three vibrational frequencies at 1985 cm^{-1} , 2016 cm^{-1} and 2047 cm^{-1} are assigned to four fundamental carbonyl stretching modes, and two of four modes are corresponding to the 2016 cm^{-1} frequency. The two lower vibrational frequencies 1985 cm^{-1} and 2016 cm^{-1} are due to the perpendicular carbonyl stretches while the highest frequency 2047 cm^{-1} is due to the coaxial carbonyl stretches. The lifetimes of these carbonyl stretching vibrations are obtained through the fit and analysis in terms of two-component exponential decays modified Gaussian function with the temporal response.

In the second and major part of the thesis (Chapter 3, 4, 5, and 6), we describe the application of ultrafast transient absorption spectroscopy to the study of photosynthetic systems. Photosynthesis is the most common light reaction process in nature, which is carried out in chloroplast. Thylakoids, which are organized into sacs of green inner membranes in the chloroplast, are membranes-bound compartments and the sites of the light reaction of photosynthesis. The light reaction function mainly depends on four major protein complexes (photosystem II, photosystem I, cytochrome b_6f complex and adenosine triphosphate synthase) which localize at the thylakoid membranes. Usually, the pigments for photosynthesis are bound in these components. The pigments absorb the light energy and transfer the excitation energy to the reaction centres which also contain the pigments.

Chlorophylls are the most important pigments in nature. They widely exist in the plant, bacterial and algae and absorb visible light in the red and blue wavelengths but reflect the green and near-green portions. The typical ultraviolet-visible absorption bands of chlorophylls are localized at two regions: the Soret Band and the Q bands. The Soret bands correspond to the S_n states of chlorophylls while the Q bands are corresponding to the S_2 and S_1 states of chlorophylls. The energy transfer dynamics between the Soret band and the Q band in chlorophyll *a* and *b* was investigated by our pump probe spectrometer. It is found that although the energy gap between the Soret band and the Q band in chlorophyll *b* is smaller than that in chlorophyll *a*, the lifetimes of the $B \rightarrow Q$ energy transfer processes in chlorophyll *b* are longer than that in chlorophyll *a*.

Most of chlorophylls in nature are bound in the light-harvesting antenna pigment-protein complex (LHC) II which is a supramolecule associated with the core of photosystem II in photosynthetic systems. LHC II exists as a trimer and plays an important role in the light absorption and the efficient electron and excitation energy transfer in photosynthetic reactions. Transient absorption measurements were carried out for three types of LHC II to study the carotenoid to chlorophyll energy transfer dynamics. The excitation energy absorbed by carotenoids is transferred to both chlorophylls via the Q_x bands of chlorophylls in a short time scale of few hundreds of femtoseconds in native and wild type LHC II, but in H120L mutant LHC II the excitation energy primarily is guided into chlorophyll *a* via the Q_x bands of both chlorophylls. The energy transfer among the chlorophylls occurs in the subsequent picoseconds and tens of picoseconds.

Photosystem (PS) I complex is an important component in photosynthetic systems. In photosynthesis, PS I absorb the light energy and supply the negative redox and transfer the excitation energy as an intermediary. The energy transfer dynamics among bulk antenna pigments of PS I excited at 650 nm was measured by our transient absorption spectrometer.

The excitation energy absorbed by chlorophyll *b* is transferred from the blue side of bulk pigments to bulk chlorophylls and red chlorophyll. The red chlorophylls absorbing the light with wavelengths at around 715 nm, 730 nm and 750 nm were observed to accept the excitation energy from the 682 nm chlorophyll pool at time scales of 1.93 ps and 10.9 ps.

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Chapter 1

Introduction

Spectroscopy is the study of the interactions of matter with electromagnetic fields [1.1]. It is a principal technique and invaluable tool to investigate the structure and dynamical processes of matter, and provides us most of the knowledge about microscopic particles [1.2]. Without this vital tool, it is very hard to imagine our microscopic understanding of nature [1.1]. According to the order of the response of matter with respect to the applied external electric field, spectroscopic techniques can be classified as linear and nonlinear spectroscopy. In linear spectroscopy, the material polarization responds linearly with the incident electric field and includes the process of one-photon absorption, reflection and refraction which are weak light-matter interactions. Most optical processes of matter involving external electric field are dominated by the nonlinear optical response and belong to nonlinear spectroscopy, such as transient absorption spectroscopy, two-photon absorption and sum frequency generation (SFG).

For the past few decades, many scientific questions about the structural and dynamical processes of different molecular systems have been solved by linear and nonlinear spectroscopy. In 1948, the hyperfine structure of hydrogen and its isotope is derived with linear absorption spectroscopy by Nafe and Nelson [1.3]. Later, the Fourier transform infrared spectroscopy (FTIR) was developed to obtain the information of vibrations of the chemical bonds of material and identify every different kind of material based on their unique infrared spectrum [1.4]. However, with the increase of strength of the applied optical field, more and more new interesting nonlinear phenomena were discovered by scientist. An

experiment was designed and performed by Franken and his co-workers in 1961 [1.5], one beam with wavelength of 694.3 nanometres (nm), pulse duration of 1 millisecond (ms) and energy of 3 joules is focused into a crystalline quartz, and then the wavelength of output beam is changed to 347.2 nm, and this new phenomenon is named second harmonic generation (SHG). Typically, nonlinear optical properties of material are observable only when the applied electric field is sufficiently intense. Massive nonlinear optical responses of different kinds of material have been investigated only after the laser was invented by Maiman in 1960 [1.6]. Henceforth, the gate of “treasure” containing nonlinear optical phenomena is formally open and a series of spectroscopic techniques are developed to study these nonlinear phenomena, for example, nonlinear Raman spectroscopy, and transient absorption spectroscopy and so on.

Furthermore, scientists discovered that some dynamical processes are extremely fast and on the short time scales of picosecond (ps), femtosecond (fs), and even attosecond (as). In order to study these quick dynamical processes, ultrashort laser pulse is utilized in spectroscopic technique and the novel technique is called ultrafast laser spectroscopy. Only with the invention of fast “camera” – ultrafast laser spectroscopy, are the researchers able to take ‘photos’ of molecules in a short time scale and obtain information of the fast dynamical processes of molecular systems. Due to the different research objects, ultrafast laser spectroscopy can be classified as ultrafast transient absorption spectroscopy, ultrafast imaging technique, and ultrafast fluorescence up-conversion technique, and so on. Recently, ultrafast optical spectroscopy has been extended from one-dimension to two-dimension, three-dimension and even four-dimension in the frequency domain [1.7]. Multidimensional optical spectroscopy provides a richer amount of information to understand the dynamical processes and expand our understanding on molecular system in a short time scale. Although multi-dimensional spectroscopy has some advantages, ultrafast transient absorption

spectroscopy is still the most common and valid method of choice to study most processes of molecular dynamics. In the following section, we will focus on the ultrafast transient absorption spectroscopy.

1.1 Ultrafast Transient Absorption Spectroscopy

Ultrafast transient absorption spectroscopy provides the information on the mechanistic and kinetic details of molecular systems and performs at a very wide range of wavelength from far-infrared to deep-ultraviolet regions. It is a four-wave-mixing technique and involves two laser pulses: one strong pump pulse and one weak probe pulse, thus this technique also can be called ultrafast pump-probe spectroscopy. Usually, the pump pulse is much stronger than the probe pulse. The pump pulse induces the electronic transitions of a certain amount of molecules in the sample when the pump pulse goes through a sample. Then, the probe pulse passes through the same part of sample [1.8, 1.9], as shown in Figure 1.1.

Figure 1.1: Schematic diagram of transient absorption spectroscopy.

In transient absorption spectroscopy, a part of the molecules in sample are pumped from their ground state to excited state by a pump pulse and a weak probe pulse is sent through the same volume of sample with a delay time T with respect to the excitation pulse. With a fixed delay

time T , before reaching the sample, the pump pulse goes through an optical chopper which blocks every other pump pulse. Thus, alternately the sample is being “pumped” and “unpumped”. A difference absorption spectrum is calculated by the following equation:

$$\Delta A(\lambda) = -\log(I(\lambda)_{\text{pumped}}/I(\lambda)_{\text{unpumped}}) \quad (1.1)$$

where $I(\lambda)_{\text{pumped}}$ is the absorption spectrum of the “pumped” sample and $I(\lambda)_{\text{unpumped}}$ is the absorption spectrum of the “unpumped” sample. By varying the delay time τ and recording the difference absorption spectrum at each time delay, a $\Delta A(\lambda, T)$ profile as a function of T and wavelength λ is obtained. Through the analysis of the $\Delta A(\lambda, T)$ result, we can get the information on the dynamical processes of the molecular systems.

Generally, the transient absorption spectrum $\Delta A(\lambda, T)$ consists of three main contributions:

- (1) Instantaneous ground-state bleaching. A fraction of the molecules at the ground state is pumped to their excited state by the pump pulse. The probe beam will be absorbed less during passing through the sample. Therefore, a negative signal will be observed in the spectral range of ground state absorption.
- (2) Stimulated emission. Some of the molecules are pumped to the excited state, and then stimulated emission to the ground state will occur when the probe pulse goes through the sample. In general, the signal from stimulated emission has the same wavelength range with the stationary fluorescence. Due to stimulated emission, the light intensity on the detector will increase and that leads to a negative signal in the wavelength region of stimulated emission.
- (3) Excited-state absorption. The molecules which are in the excited state may absorb some photons from the probe beam to higher excited state in certain wavelength ranges and that leads to a positive signal at these wavelengths.

Apart from the three main contributions, for some photobiological or photochemical systems, new photoproducts absorption can also contribute the spectrum $\Delta A(\lambda, T)$. After the excitation pulses, the molecules may be in a transient or long-lived molecular state due to photoreactions and the absorption of such photoproducts will supply a positive signal to the $\Delta A(\lambda, T)$ spectrum [1.8, 1.10].

Since ultrafast transient absorption spectroscopy was developed to study the dynamical processes on extremely short time scale (ns to as), this technique has been utilized to investigate massive interesting scientific questions which extend our knowledge on molecular scale. In 1984, a picosecond laser with pulse duration of 3 ps was used to study the dynamics of stilbene and aniline at temperature lower than 5 K by Zewail's group [1.11]. Through this experiment, they probed the effects of rotations and obtained the lifetime of the first singlet state of stilbene and aniline in gas phase. Subsequently, femtosecond lasers were used in pump-probe experiments, such as Engel and Metiu who investigated the predissociation of NaI using a 50 fs pulse [1.12]. In order to probe reaction dynamics occurring at a much shorter time scale, the attosecond lasers are utilized in transient absorption spectroscopy. For example, Okin et al have utilized the attosecond pump-probe spectroscopy to image molecular Coulomb explosions [1.13].

Amongst the numerous molecules or molecular complexes, we are interested in two different kinds of molecular systems, metal carbonyl complexes and photosynthetic pigment-protein complexes. Metal carbonyl complexes are of great significance as catalyst in organic synthesis [1.14] and also useful in the preparation of thin metal films [1.15]. Thus the detailed studies of the dynamical chemical properties of these complexes are necessary. Photosynthetic complexes are vital for all the lives in the earth. The photosynthesis produces most of the chemical energy and oxygen on Earth by means of light energy with carbon dioxide, water. During the photosynthesis, different dynamical processes including internal

conversion, intersystem crossing, and fluorescence together occur in the photosynthetic complexes [1.10]. Therefore, the detailed dynamical information should be clarified. For illuminating the dynamical properties of the above two complexes, we will utilize the ultrafast transient absorption spectroscopic technique to study the vibrational relaxation dynamics of carbonyl stretching modes in a metal carbonyl complex and the dynamical processes of photosynthetic pigment-protein complexes.

1.2 Organization of Dissertation

In chapter 2, we report on the study of the vibrational relaxation dynamics of carbonyl stretching modes in $\text{Mn}_2(\text{CO})_{10}$ solution is investigated by using broad-band infrared pump-probe spectroscopy.

In chapter 3, we briefly introduce the structures and functions of photosynthetic systems including chloroplast, photosystem I, photosystem II, light-harvesting complex II and pigments. We also describe the non-collinear pump probe spectrometer built to study the dynamics of photosynthesis.

In chapter 4, we report on the study of the non-radiative relaxation of chlorophylls excited at the Soret absorption spectral range.

In chapter 5, we use femtosecond transient absorption spectroscopy to study the carotenoid to chlorophyll energy transfer dynamical processes in three types of LHC II (native, wild and h120l mutant LHCII).

In chapter 6, the energy transfer dynamics among bulk antenna pigments of PS I excited at 650 nm was measured and the red chlorophylls in PS I were detected by using femtosecond pump probe spectroscopy.

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Chapter 2

Ultrafast Carbonyl Stretching Vibrational Dynamics in $\text{Mn}_2(\text{CO})_{10}$

2.1 Introduction

In general, the vibrational frequencies of stretching bonds in molecules are located at the infrared range [2.1, 2.2]. In order to detect the fast dynamics of stretching bonds, an ultrafast infrared laser should be used as the light source in ultrafast transient absorption spectrometer. Generally this technique is called ultrafast infrared pump probe spectroscopy. Ultrafast infrared pump probe spectroscopy is a frequently used technique to study molecular vibrational relaxation dynamics, especially in the case of solutions or other condensed phase, where nonequilibrium vibrational energy distributions of the solute molecules will decay rapidly to equilibrium due to the energy being transferred into the numerous motions of the solvent [2.3]. The lifetime of relaxation process is usually around hundreds of femtoseconds or a few of picoseconds [2.2].

In the past few decades, interesting research about vibrational dynamics of chemical bonds with ultrafast infrared pump probe spectroscopy has been performed. The OH-stretching frequency of HOD in liquid D_2O has been studied with femtosecond infrared spectroscopy by Tokmakoff's group. They found an underdamped oscillation with a period of 0.17 ps and the lifetime of vibrational correlation decay is around 1.2 ps in the hydrogen-bond [2.4]. Another example is that the carbon-hydrogen bonds can be activated by some certain metal complexes in room temperature, in this case, Bromberg and his co-workers have used time-resolved

infrared spectroscopy to generate a detailed picture of the C-H activation reaction and thereby identify all the intermediate structures in the reaction of $\text{Tp}^*\text{Rh}(\text{CO})_2$ in alkane solution [2.5].

In nature, the carbon-oxygen bond is one of the most common bonds in the molecules and has important effects on the molecular properties, especially in photoactive transition metal carbonyl complexes which can play an important role as catalysts [2.6]. The metal carbonyls are also often used in carbonyl photo-addition reactions [2.7, 2.8]. To understand the reaction mechanisms of the metal carbonyls which typically include photo-induced structural rearrangement processes, the study of vibrational dynamics of the metal carbonyls is of great importance.

Cotton and Kraihanzel developed a model to analyse and assign the infrared-active carbonyl stretching frequencies of some metal carbonyls. This model is applied to the different metal carbonyls including $\text{Cr}(\text{CO})_6$, $\text{Mo}(\text{CO})_6$ and $\text{W}(\text{CO})_6$. The results from this model for these compounds are in agreement with the valence bond theory and supported by Raman spectra [2.9]. The spectroscopic technique has been applied to study the carbonyl stretching absorptions for $\text{Mn}(\text{CO})_5\text{H}$, $\text{Mn}(\text{CO})_5\text{D}$, $\text{Re}(\text{CO})_5\text{H}$ and $\text{Re}(\text{CO})_5\text{D}$, and the assignment of carbonyl modes of the main metal carbonyls was reported in the region of $1900\text{--}2200\text{ cm}^{-1}$. Through exchange with ^{13}CO the CO modes were experimental verified by Braterman's group [2.10].

With the development of ultrafast laser spectroscopy, the study of the fast vibrational relaxation dynamics of the metal carbonyls can be achieved. In 1994, Fayer et al performed picosecond infrared pump-probe experiments to investigate the lifetime of vibrational relaxation of metal carbonyl solutes in different temperatures. They found the vibrational lifetime decrease with temperature for $\text{Cr}(\text{CO})_6$ and $\text{W}(\text{CO})_6$ dissolved in tetrachloride and with the increase of temperature the lifetime of vibrational relaxation of $\text{W}(\text{CO})_6$ in

chloroform becomes longer while the lifetime of $\text{Cr}(\text{CO})_6$ slightly decreases [2.11]. The two-dimensional (2D) infrared spectroscopy also was widely utilized to study the vibrational relaxation dynamics of the various metal carbonyl complexes, Kubarych et al mentioned that metal carbonyl complexes are ideal systems for enhancing the versatility of 2D infrared spectroscopy [2.12]. Recently, the vibrational relaxations dynamics of $\text{Os}_3(\text{CO})_{12}$ has also been studied with ultrafast infrared pump-probe experiment by Yan et al, and from transient absorption spectra and kinetic traces the properties of the four carbonyl stretching modes has been obtained [2.13].

In the organic synthesis, $\text{Mn}_2(\text{CO})_{10}$ is widely used as an important catalyst [2.14, 2.15]. In the visible range, two absorption maxima with wavelength of 780 nm and 480 nm is observed [2.16]. There are three fundamental vibrational modes corresponding to the carbonyl stretch vibration in the spectral region $1900\text{-}2200\text{ cm}^{-1}$ [2.17]. In this chapter, we will study the vibrational relaxation dynamics of the CO stretch vibration in $\text{Mn}_2(\text{CO})_{10}$ by using femtosecond transient absorption spectroscopy.

2.2 Material and Method

2.2.1 Femtosecond Infrared Pump Probe Spectrometer

In this experiment, the laser systems which is used in ultrafast infrared pump-probe spectrometer contain a regeneratively Ti:Sapphire amplifier (Coherent, Legend Amplifier, 1 kHz, 800 nm, 140 fs) with a Q-switched pump laser (Coherent, Evolution, 1 kHz, 527 nm) and an oscillator (Coherent, Vitesse, 80 MHz, 800 nm, 140 fs) which provides the seed laser. The laser power from Legend amplifier is around 1 W, and it is used to pump an optical parametric amplifier (OPA) (Coherent, Opera, type II, 4 mm $\beta\text{-BaB}_2\text{O}_4$). Through the OPA, the 800 nm pulse is converted to two near-infrared pulses termed signal pulse and idler pulse.

Both of the signal and idler pulses are then introduced to a difference frequency generation stage (DFG) (Coherent, DFG, type II, 2 mm AgGaS₂). Tunable mid-infrared laser pulse (2.5 to 10 μ m) with a repetition rate of 1 kHz is generated by adjusting the crystal angles for phase matching in the β -BaB₂O₄ (BBO) and AgGaS₂ (AGS) crystals.

Figure 2.1: Schematic representation of ultrafast transient absorption spectrometer. SM1: Concave Mirror, $f = 100$ mm; SM2: Concave Mirror, $f = 150$ mm; Ge: Germanium Window; Ir1 and Ir2: Iris; Wd: CaF₂ Wedge Window; BS: CaF₂ Beam Splitter, 50:50; L1, L2, L3 and L4: Lens, $f = 150$ mm, 75mm, 75mm, 75 mm; Chp: Chopper; Pol: BaF₂ Wire Grid Polarizer; PM1 and PM2: Parabolic Mirror, $f = 160$ mm.

The setup of ultrafast infrared pump-probe spectrometer is represented in Figure 2.1. Because the infrared light is invisible, a He-Ne Laser (632.8 nm) is used to find the position of infrared beam and thereby align the infrared beam. The beam generated in the DFG unit is split into two parts by using a 50:50 CaF₂ beam splitter. The transmitted beam is used as the reference pulse. The reflected part further is then separated into two beams with a CaF₂ wedge window. After the wedge window, the stronger transmitted beam is used for pump light and the weaker beam as probe light. The energy ratio should be higher than 18:1 between the pump and probe. The pump beam is chopped at a frequency of 500 Hz. After the

laser beam go through the splitter and wedge window, the energy of pump pulse is estimated to be 30% of the energy of the output pulse from DFG.

Both the pump and probe pulses are focused and overlapped in the sample by an off-axis parabolic mirror (focal length 160 mm). The beam diameter at the focal point D_f is defined as the diameter where the intensity is $1/e^2$ of the intensity of a Gaussian beam, and so D_f can be estimated by the equation $D_f = 4 \cdot F \cdot \lambda / (\pi \cdot D_i)$, where F is the focal length of the parabolic mirror and D_i is the diameter of the initial and λ is the centre wavelength. In order to get the spatial overlap of the pump and probe pulses, we put a pin hole (50 μm in diameter) at the focal point and then align the two beams through pin holes simultaneously.

After the focal point, the pump light is blocked and the probe light is re-collimated by another off-axis parabolic mirror. Thereafter the probe beam is directed into a monochromator (Dongwoo Optron Co, DM320i, $f = 320$ mm, two 150 lines/mm grating blazed at 4.0 μm and 6.0 μm) which has a resolution of 2 cm^{-1} , and detected by a liquid nitrogen cooled InSb detector (Infrared Associates, Inc.). The polarization of the pump pulse is set to the magic angle of 54.7° by the placement of wire-grid BaF_2 polarizer to measure the pure population dynamics without the anisotropy-changing effects from molecular structural changes and rotational diffusion.

Finally, the signal is sent to an integration boxcar. In the boxcar, the signal intensity is normalized with the reference pulse which is detected by another InSb detector. In this way, we can eliminate the fluctuations in the signal caused by the laser instability. Furthermore, the signal is subsequently amplified in a lock-in amplifier and the data is collected by NI BNC-2100 data acquisition card.

2.2.2 Characterization of the Laser Pulses

Figure 2.2: The spectrum of laser pulse used in pump-probe experiment.

The centre frequency of laser pulse was set to 2024 cm^{-1} with a spectral full width at half maximum (FWHM) of 100 cm^{-1} , as shown in Figure 2.2. The power of the generated mid-infrared pulse from DFG was measured to be 3.6 mW and so the energy of pump pulse at the focal point should be around $1.08 \text{ }\mu\text{J}$. The beam diameter of the mid-infrared pump light at the focal point D_f was calculated to be around 0.1 mm and the fluence of pump pulse is estimated to be 10 mJ/cm^2 .

Figure 2.3: Cross-correlation measurement for the laser pulse and the fit by Gaussian function.

A second harmonic generation cross-correlation setup based on a LiIO_3 crystal (0.4 mm, type I) was used to measure the mid-infrared pulse duration. The result with Gaussian function fitting is represented in Figure 2.3. The FWHM of cross-correlation is 290 fs corresponds to the pulse duration of 205 fs (assuming a Gaussian temporal shape).

2.2.3 Sample Preparation

$\text{Mn}_2(\text{CO})_{10}$ powder and n-hexane solvent were purchased from Sigma-Aldrich. All the chemicals were directly used to make the solution without further purification. The solution was prepared to be around 1 mM resulting in a maximum static absorbance of about 1 OD at the strongest absorption band of $\text{Mn}_2(\text{CO})_{10}$. The sample was put into a transmission cell which comprises of two 2 mm thick CaF_2 windows separated by a Teflon spacer of 200 μm thickness.

2.2.4 Structure of $\text{Mn}_2(\text{CO})_{10}$

Figure 2.4: Structure of $\text{Mn}_2(\text{CO})_{10}$. Red: Oxygen; Grey: Carbon; Purple: Manganese.

As shown in Figure 2.2, the structure of $\text{Mn}_2(\text{CO})_{10}$ is an uncommon symmetry, and consists of two subunits $\text{Mn}(\text{CO})_5$. These two subunits are staggered in the stable rotamer. According to the axis of Mn-Mn bond (293 pm), there are two types of CO ligands in the molecule, one CO on each manganese is coaxial and the other four are perpendicular. Overall, the molecule belongs to the D_{4d} point group [2.18].

2.3 Theoretical Calculation

We used Gaussian 09 to calculate the infrared absorption spectrum of $\text{Mn}_2(\text{CO})_{10}$. Through the calculation, we are only able to observe four vibrational modes from CO stretches and the other CO stretching modes disappear because of molecular symmetry. The four existing modes are labelled as mode 1, mode 2, mode 3 and mode 4, which are shown in Figure 2.3. These four modes can be divided into two groups. Mode 1, 2 and 3 are exclusively from the

local modes of the perpendicular CO stretches and mode 4 belongs to the coaxial CO stretches.

Figure 2.5: The four carbonyl stretching modes of $\text{Mn}_2(\text{CO})_{10}$ which can be observed in the infrared range.

Figure 2.6: Infrared absorption spectrum calculated by Gaussian 09.

However, when we check the infrared absorption spectrum of the calculation result, only three peaks are observed in the infrared range, as shown in Figure 2.4. The three peaks are marked as peak α corresponding to 2071 cm^{-1} , and peak β at 2157 cm^{-1} , and peak γ with 2209 cm^{-1} . The peak β is obvious higher than the other peaks.

The calculation result also shows the vibrational energies of four CO stretching modes. Compare the energies of four vibrational modes with the peaks of infrared absorption spectrum; we discover that the mode 2 and mode 3 own the same vibrational energy at peak β 2157 cm^{-1} , and mode 1 has the lowest energy corresponding to peak α 2071 cm^{-1} , and mode 4 possesses the highest energy at peak γ 2209 cm^{-1} .

2.4 Results and Discussion

2.4.1 Linear Infrared Absorption Spectrum

The linear infrared absorption spectrum of $\text{Mn}_2(\text{CO})_{10}$ in hexane was measured at room temperature and is presented in Figure 2.7. There are three absorption bands observed around 2000 cm^{-1} and the three peaks are assigned to the fundamentals in the CO stretching region for $\text{Mn}_2(\text{CO})_{10}$ in solution [2.17]. The three absorption bands with frequencies peaked at 1984 cm^{-1} , 2015 cm^{-1} and 2046 cm^{-1} are labelled as a, b and c respectively. Both absorption bands a and b are normal modes whose components are from the perpendicular CO stretching modes, and the other band c also is normal mode whose components are from the coaxial CO stretching mode. The three fundamental CO stretching frequencies of $\text{Mn}_2(\text{CO})_{10}$ can be covered perfectly by the laser spectrum illustrated in Figure 2.2.

Compare the FTIR spectrum with the calculation spectrum, we can find that the three bands have large red shifts. Between band a of the FTIR spectrum and peak α of calculation spectrum, the energy is shifted from peak α 2071 cm^{-1} to band a 1984 cm^{-1} with the energy

difference 87 cm^{-1} . Peak β at 2157 cm^{-1} is 142 cm^{-1} greater than band b at 2015 cm^{-1} . The energy gap between peak γ (2209 cm^{-1}) and band c (2046 cm^{-1}) is the biggest at about 163 cm^{-1} . A possible for the systematic differences could be that the calculation results were carried out at 0 K and in the gas phase.

Figure 2.7: Ground state linear absorption spectrum of $\text{Mn}_2(\text{CO})_{10}$ solution in n-hexane. Three main bands are labelled as a, b and c with frequencies 1984 cm^{-1} , 2015 cm^{-1} , and 2046 cm^{-1} , respectively.

In summary, band a is from the vibrational mode 1 of perpendicular carbonyl stretches, with the band b corresponding to the perpendicular carbonyl stretching mode 2 and mode 3 whose vibrational energies are same, and band c is from mode 4 of coaxial carbonyl stretches.

2.4.2 Transient Absorption Spectra

Transient absorption spectra were taken in the frequency range from 1950 to 2075 cm^{-1} at different time delays and the result is shown in Figure 2.8. From the result, we can see there

is no obvious time-dependent change of shape and position to the peaks except band b decays faster than other bands. The three negative bands at 1985 cm^{-1} , 2016 cm^{-1} and 2047 cm^{-1} are labelled as a, b and c and nominally assigned to the three fundamental transitions ($\nu_0 \rightarrow \nu_1$) of the CO stretches consistent with the linear absorption spectrum in Figure 2.7. The three positive bands labelled as d, e and f whose centre frequencies are 1975 cm^{-1} , 2011 cm^{-1} and 2043 cm^{-1} , respectively, are assigned as the overtone transition ($\nu_1 \rightarrow \nu_2$), with overtone anharmonic shifts of $\Delta_{ad} = 10\text{ cm}^{-1}$, $\Delta_{ae} = 5\text{ cm}^{-1}$, and $\Delta_{cf} = 4\text{ cm}^{-1}$, respectively. The positions and relative intensities of these positive and negative bands will be discussed in the Section 2.8.3.

Figure 2.8: Transient absorption spectra at different time delays 0 ps, 80 ps, 200 ps. Bands a, b and c are the three fundamental transitions at 1985 cm^{-1} , 2016 cm^{-1} , and 2047 cm^{-1} , respectively. Bands d, e and f are the individual overtone transitions centred at 1975 cm^{-1} , 2011 cm^{-1} , and 2043 cm^{-1} , respectively.

2.4.3 Pump Probe Dynamics Traces

Figure 2.9: Frequency-resolved pump-probe traces with numerical fits. Left part: traces A, B and C are the kinetics of fundamental transitions at 1985 cm⁻¹, 2016 cm⁻¹, and 2047 cm⁻¹, respectively. Right part: traces D, E, and F are the kinetics of overtone transitions at 1975 cm⁻¹, 2011 cm⁻¹, and 2043 cm⁻¹, respectively.

All the three absorption bands due to carbonyl stretching modes around 2000 cm⁻¹ were simultaneously excited by the broad-band pump pulse with centre frequency 2024 cm⁻¹ and bandwidth of 100 cm⁻¹, and Figure 2.9 shows the kinetic traces recorded at 1985 cm⁻¹, 2016 cm⁻¹, 2047 cm⁻¹, 1975 cm⁻¹, 2011 cm⁻¹ and 2043 cm⁻¹ and labelled as traces A, B, C, D, E and

F, corresponding to band a, b, c, d, e and f, respectively. Traces A, B and C belong to the three fundamental transitions of carbonyl stretches, and traces D, E and F are attributed to the overtone transitions. All the traces were fitted with the exponential modified gaussian function with the temporal response (205 fs). The coherent artifact from coupling between the pump and probe pulses is observed near zero time delay and it is independent of the dynamics of the carbonyl stretching mode.

In Figure 2.9, traces A, B and C have qualitatively similar features and all of the three traces can be fitted well with a bi-exponential decay function. From the fitting, we can know trace A consists of a fast decaying component τ of $2.8(\pm 2)$ ps and a slow decaying component T of $550(\pm 50)$ ps, trace B with a fast decaying component τ of $1.0(\pm 0.5)$ ps and a slow decaying component T of $275(\pm 125)$ ps, and traces C with a fast decaying component T of $1.7(\pm 0.2)$ ps and a slow decaying component T of $183(\pm 1.4)$ ps.

The kinetic traces measured at the overtone transitions frequencies also describe the population relaxation dynamics of the first excited vibrational state. Traces D, E and F in Figure 2.9 are the pump probe traces for the three overtone transitions which are labelled as d, e and f in Figure 2.8. All the kinetic traces from the overtone transitions were fitted with the same procedure as described above. The fitting traces for the overtone transition show the results: trace D is well fitted by a function consisting of a fast decay τ of $2.6(\pm 0.6)$ ps and a slower decay T of $145(\pm 28)$ ps, trace E are fitted with a fast decay τ of $0.4(\pm 0.1)$ ps and a slower decay T of $227(\pm 2)$ ps, and trace F with τ of $1(\pm 0.1)$ ps and T of $251(\pm 3)$ ps.

2.4.4 Fit of Transient Absorption Spectra

For the molecular system with three observable fundamental transitions, there should be nine overtone transitions with three transitions from individual bands and six transitions from combination bands in principle. This is illustrated by means of double side Feynman

Diagrams in Figure 2.10, and the absorption bands for these overtone transitions may be overlapped with the fundamental transitions. To obtain real vibrational population dynamics of the three absorption bands, the positions and relative intensities of the three bands need to be identified. The transient absorption spectrum taken at a time delay of 80 ps was fitted with a series of Gaussian functions, as shown in Figure 2.11. The fit was optimized with the fixed spectral positions of the fundamental and overtone transition bands to get the best result. The result with all the parameters is listed in Table 2.1. From the fit result, we can know the bandwidth of the individual bands is in the range from 2.9 to 10.3 cm^{-1} , and the assignments of the fundamental and overtone bands are straightforward, and the assignments for combination bands are unobvious.

Figure 2.10: Double side Feynman diagrams of all the transitions. Type I: the three fundamental transitions: I_a, I_b, and I_c. Type II: the three overtone transitions from individual bands: II_{2a}, II_{2b}, and II_{2c}; the six overtone transitions from combination bands: II_{ab}, II_{bc}, II_{ca}, II_{ac}, II_{ba}, and II_{cb}.

Figure 2.11: Transient absorption spectrum measured at 80 ps (black empty circle) and the fit which is used by a series of Gaussian functions (red solid line).

Bands	a	b	c	d	e	f
Anharmonicity (cm^{-1})	--	--	--	-10	-5	-4
Frequency (cm^{-1})	1985	2016	2047	1975	2011	2043
Amplitude (a.u.)	0.142	0.193	0.118	-0.022	-0.273	-0.068
FWHM (cm^{-1})	2.9	7.3	4.1	8.6	10.3	8.3

Table 1: Parameters of positions, relative amplitude and FWHM used to fit the transient absorption spectrum at the delay of 80 ps.

2.5 Conclusions

In this chapter, we have used ultrafast mid-infrared pump probe spectroscopy to investigate the vibrational relaxation dynamics of the carbonyl stretching modes of $\text{Mn}_2(\text{CO})_{10}$ in hexane. In this molecule, three absorption bands can be detected. Two absorption bands are normal modes whose components are exclusively from the local modes of the perpendicular carbonyl stretches and the components of the left absorption band are exclusively from the local modes of the coaxial carbonyl stretches.

The transient absorption spectra at different time delay were obtained and analysed. The lifetimes of the vibrational absorption bands were obtained by fitting the kinetics traces. No clear indication of any direct intra-molecular energy transfer among the carbonyl stretching vibrational modes was observed by using ultrafast transient absorption spectroscopic technique.

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Chapter 3

Introduction to Photosynthetic Systems and the Visible Pump-Probe Spectrometer

3.1 Introduction

In Chapter 2, we have discussed that transient absorption spectroscopic technique was applied to study the carbonyl stretching dynamics of $\text{Mn}_2(\text{CO})_{10}$. An ultrafast infrared laser was utilized because the typical frequencies of carbonyl stretching vibrations of $\text{Mn}_2(\text{CO})_{10}$ are in the infrared energy range. However, the most common light absorption processes in nature happen in the visible range, and photosynthesis is a vital part of them.

For all the life upon the earth (except for chemolithotrophic prokaryotes), photosynthesis is the most important source of oxygen and energy [3.1]. During the photosynthetic process, about 1% of the energy from sunlight reaching the earth each day is captured by plants, algae, or bacteria, and the energy is then transduced into chemical energy by synthesizing carbohydrate compounds and oxygen from CO_2 and H_2O and/or other inorganic compounds [3.2]. Finally the oxygen and organic compounds can then be used to sustain for the animals, plants and other forms of life on Earth. In short, photosynthesis can be considered to be the most important biological process [3.3].

3.2 Photosynthetic Systems

We know that the photosynthesis process is carried out by a special organelle named chloroplasts, as shown in Figure 3.1. Chloroplasts are quite large (usually 5-10 μm) and are

characteristic of high concentration of chlorophyll, which is different from the other two types of plastids containing little chlorophyll which does not participate in the photosynthetic process [3.1, 3.4]. In the chloroplast, green inner membranes are organized into sacs, which are called thylakoids. When numerous thylakoids are placed on the stack, they are then known as the grana [3.4].

Figure 3.1: Left: Electron micrograph of a chloroplast; Right: Schematic diagram of a chloroplast [3.1].

Figure 3.2: The structures of thylakoid membrane with the four major protein complexes involved in the photosynthetic process [3.6].

Within the chloroplasts in the leaf cells, all the light-absorbing and energy-transducing functions are localized in the thylakoid membranes in the form of four major protein complexes which are embedded in the thylakoid membranes [3.5]. The four complexes are listed as follows: photosystem I (PS I), photosystem II (PS II), cytochrome b₆f complex and adenosine triphosphate (ATP) synthase, as presented in Figure 3.2.

All the four protein complexes together carry out the light-driven reactions of photosynthesis. PS I and II are very large complexes consisting of numerous pigments and are the primary electron donors in the photosynthetic process. The two photosystems carry out the light absorption and the transfer of energy and electrons, and operate with a high quantum yield [3.6]. The main difference between PS I and PS II is that PS I optimally absorbs photons with wavelength of 700 nm and PS II optimally absorbs at 680 nm. Generally, in the photosynthetic process the cytochrome b_6f complex mediates electron transport between PS II and PS I and converts the redox energy to generate the proton gradient. The function of ATP synthase is in producing ATP at the expense of the proton motive force formed by the proton gradient [3.7]. In the following sections, we will focus on the brief introduction of PS I and II.

3.2.1 General Structure of Photosystem I

In the light-driven reaction of photosynthesis, PS I is the second photosystem although the complex is named as PS I [3.8]. There are two different forms of PS I, one is the monomeric form surrounded by light-harvesting complex I (LHC I) and they exist in plants. The other one is the trimeric form which exists in cyanobacteria, as shown in Figure 3.3. The PS I in plants consists of more than 14 different subunits, whereas one monomeric unit of trimer PS I in cyanobacteria contains 12 proteins.

In the centre of both forms of PS I, a joint reaction centre is formed by two main subunits (PsaA and PsaB) which are composed of the binding of a primary electron donor (P700) and five electron acceptor (A0, A1, Fx, Fa, and Fb) and other small subunits. The subunits PsaA and PsaB contain approximately 100 chlorophyll *a* molecules and 12-16 β -carotene molecules, and these antenna pigments absorb the photons from incident light and transduce the energy to the electron donor P700 [3.9].

In the monomeric unit of cyanobacterial PS I, there are seven small subunits surrounding the reaction core: PsaF, PsaI, PsaJ, PsaK, PsaL, PsaM and PsaX, and the stromal hump is formed by three stromal proteins (PsaC, PsaD and PsaE) [3.10]. However, at least four additional subunits (PsaG, PsaH, PsaO and PsaN) instead of PsaM and PsaX exist in plant PS I [3.10, 3.11].

Figure 3.3: The structural model of plant PS I. a: Plant PS I [3.11]; b: Trimeric PS I from cyanobacterial [3.10].

3.2.2 General Structure of Photosystem II

PS II is a large membrane protein complex and one of the most vital enzymes as it produces all the oxygen in the atmosphere [3.10], as presented in Figure 3.4. PS II comprises around 20 subunits, and the reaction centre is composed of D1 (PsbA) and D2 (PsbD), and two antenna proteins CP43 (PsbB) and CP47 (PsbD), which are flanked on both sides of PS II [3.12]. Similar to PS I, a PS II contains a core antenna consisting of 35-36 chlorophylls which produce an electron donor P680. However, most of the chlorophylls in PS II are found in LHC II which are trimers of the light-harvesting proteins Lhcb 1, Lhcb 2 and Lhcb3. These Lhcb proteins are composed of 12-14 chlorophyll *a* and *b* molecules and less than 4

carotenoids [3.6]. During the photosynthetic process, in photosystem II the energy of light is captured by LHC IIs and transferred into the reaction centre (D1, or D2, or CP43, or CP47) via the minor light-harvesting proteins Lhcb6 (CP24), Lhcb5 (CP26) and Lhcb4 (CP29).

Figure 3.4: The structure of PS II [3.6].

3.2.3 Light-Harvesting Complex

In the previous section, we have mentioned that in the photosynthetic process, the first step is the collection of energy from light and then transferring it to the reaction centre of PS I and PS II. This step involves light harvesting complexes (LHC) which are comprised of light harvesting pigments bound by LHC proteins.

In higher plants, LHC usually have two types: LHC II and LHC I. The three major light harvesting proteins of Lhcb1, Lhcb2 and Lhcb3 associated at the periphery of PS II of higher plants binding the photosynthetic pigments comprise LHC II. The other minor light harvesting proteins including CP24, CP26 and CP29 are more closely associated with PS II than the major LHC II proteins. Because the three minor LHC IIs are of much lower abundance than the major LHC II, only the major LHC II is considered in the present study [3.13, 3.14]. In comparison with LHC II, LHC I is associated with PS I and the composition

and structure of LHC I is not very clear. For LHC I, the light harvesting proteins Lhca1 and Lhca4 are considered to dimerize into LHC I-730, and the proteins Lhca2 and Lhca3 dimerize into LHC I-680, and the gene products from the left two proteins Lhca5 and Lhca6 have not been identified [3.15, 3.16]. Thus, in the following section, we will focus on the LHC II.

Figure 3.5: The structure of trimeric LHC II (a) and monomeric LHC II (b: the stromal side, c: the luminal side) from spinacia oleracea. Yellow: lutein; Blue: chlorophyll *b*; Green: chlorophyll *a*; Orange: neoxanthin; Magenta: xanthophyll-cycle carotenoids. [3.18]

LHC II is a multifunctional complex. Besides light absorption and transferring energy, LHC II can also protect the photosynthetic systems from damage when there is too much light through nonphotochemical quenching which dissipate excess excitation energy [3.17]. LHC II exists as a homo-trimer or hetero-trimer. In Figure 3.5, the structure of trimeric LHC II from spinacia oleracea is shown and we can see that this trimeric LHC II is composed of three monomeric LHC IIs which are products of Lhcb1, Lhcb2 and Lhcb3 genes, respectively. Each monomeric LHC II binds 8 chlorophyll *a* molecules (a602, a603, a604,

a610, a611, a612, a613, a614), 6 chlorophyll *b* molecules (b601, b605, b606, b607, b608, b609) and four carotenoids (Lut620, Lut621, Neo, Xanc) [3.18, 3.19].

3.2.4 Chlorophylls and Carotenoids

Figure 3.6: The molecular structure of chlorophyll *a*, chlorophyll *b* and lutein.

Photosynthesis depends on the photoreactivity of pigments. In land plants, these pigments usually include chlorophyll *a*, chlorophyll *b* and carotenoids. Both chlorophylls are composed of magnesium-containing substituted tetrapyrroles and a long alcoholic carbon chain, as presented in Figure 3.6. Chlorophyll *b* differs from chlorophyll *a*, in that the C7 methyl substituent in chlorophyll *a* is replaced by a formyl group in chlorophyll *b*. The typical

ultraviolet-visible absorption frequencies of chlorophylls are in two regions: the Soret or B Band (400-500 nm) and the Q bands (550-700 nm) [3.20]. The carotenoids have similar electronic states because they have a similar π -electron conjugated backbone and the structure of lutein which is a kind of carotenoids is shown in Figure 3.6. Both of the ground state and first singlet state are A_g states, so transitions between them are forbidden by the selection rule. Thus, the allowed absorption spectrum appears between 400 and 550 nm due to the second excited state [3.21].

3.3 Introduction to Research in Photosynthetic Systems

Photosynthesis is always a hot research topic since the 20th century and has attracted many groups' attention, and a number of experiments were done. In the early 1900s, the relation between the development of chlorophyll and the beginning of photosynthetic process has been investigated by Irving [3.22]. Emerson studied the effect of different concentrations of chlorophyll on the rate of photosynthesis [3.23]. Osterhout and Haas tried to discover the secrets of photosynthesis by controlling temperature, pH and concentrations of CO₂ [3.24].

With technological development, scientists start to apply the spectroscopic techniques to study the mechanism of photosynthesis. The linear absorption spectra and decay kinetics of chlorophyll *a* and *b* were studied at room temperature by flash-illumination apparatus by Linschitz and Sarkanen, and they got the accurate absorption spectra and very rough results of lifetimes of chlorophyll *a* and *b* in 1958 [3.25]. Berthomieu et al used FTIR spectroscopy to characterize the vibrational absorption spectra from CO stretching mode of the primary quinone acceptor in PS II and assign the positive signal at 1478 cm⁻¹ to the C-O stretching

mode and the signals at 1645 cm^{-1} and 1630 cm^{-1} to the C=O modes and the signal at 1658 cm^{-1} to a δNH_2 histidine mode [3.26].

However, due to the ultrafast process of energy transfer among the pigments in the photosynthetic process, a much faster “camera” is necessary for investigating the dynamics of the photosynthetic systems. Wasielewski’s group applied picosecond transient absorption spectroscopy to measure the rate of formation of the primary electron donor P700 with the primary electron acceptor A_0 in 1986, and the signal was recorded as a function of time with 1.5 ps time resolution. They found that the lifetime of the bleaching at 690 nm is around 13.7 ps [3.27]. Later, Bittner et al apply femtosecond transient absorption spectroscopy to study the excitation energy transfer dynamical processes of the LHC II from spinach at room temperature and 12 K. They illustrated that the energy transfer between chlorophyll *b* and chlorophyll *a* occurs on both subpicosecond and picosecond time scales [3.28].

Ultrafast transient absorption spectroscopy is a proper method to study the fast dynamical processes of the photosynthetic systems, because the dynamical processes of photosynthesis are at short time scale (ps, fs or as); In the next section, we will describe our pump probe spectrometer which we use to study the dynamics of photosynthesis.

3.4 The Setup of Our Pump-Probe Spectrometer

3.4.1 The Laser System

A mode-locked Ti:Sapphire oscillator (Coherent, Vitesse 800) is used to generate the laser pulses with a temporal width of 120 fs (FWHM), a spectral bandwidth of approximately 8 nm around 800 nm mid-wavelength and at a repetition rate of 80 MHz. The output power is 0.2 W and the energy of single pulse is 2.5 nano-joules (nJ), thus this laser is not suitable for our pump-probe measurements and must be amplified.

The laser from mode-locked oscillator is used as the seed laser and directed into a regenerative Ti:Sapphire amplifier (Coherent, Legend). In the amplifier, the seed laser is stretched temporally by a pulse stretcher to prevent the high peak power associated with a non-stretched pulse which may damage the amplifier medium. The Ti:Sapphire crystal in the amplifier is excited to the upper state by a Q-switched pulsed laser with wavelength of 527 nm, power of 8 W and repetition rate of 1 kHz (Coherent, Evolution 15). The amplified laser is then compressed by a pulse compressor. Finally, the power of output laser can reach around 1 W (1 mJ per pulse), and the wavelength of 800 nm, and the pulse duration is 140 fs which is a bit longer than the seed laser and the repetition rate is now at 1 kHz.

Part of the amplified output laser beam (400 mW) is used to pump a commercial non-collinear optical parametric amplifier (NOPA) from Light Conversion Ltd. The wavelength of output beam from NOPA is tunable, the range is from 500 nm to 750 nm and 850 nm to 1000 nm. This light is used as the pump beam. A small fraction (less than 2 mW) of the 800 nm laser is focused into a 2 mm sapphire window to generate white-light continuum between 450 nm and 750 nm. This white-light continuum usually is used as the probe beam.

3.4.2 Other Components of the Pump-Probe Spectrometer

During the pump-probe measurements, an important factor is the time delay between the pump pulse and the probe pulse, which must be controlled accurately. In our setup, we will use the delay stage from Physik Instrumente (PI) GmbH & Co. KG and a broadband hollow retroreflector which is fixed on the delay stage. When the laser is reflected by the hollow retroreflector, the position of the output beam must not be changed. This is ensured by a sensor with resolution of 10 μm . The sensor is put far away from the retroreflector (more than 10 m) and used to detect the position of the reflected beam. The delay stage will be moved from one end to the other end. During the moving, the position of the reflected beam will be

recorded. Through adjusting the input beam, we make sure that the beam position has no change during the delay stage is being moved. At last, at least two irises should be put and fixed to record the correct light path.

Figure 3.7: Schematic representation of alignment of delay stage.

Figure 3.8: The calibration for CCD array in our setup.

In our setup, the probe beam is dispersed in a monochromator (HORIBA Jobin Yvon, TRIAX 190) and detected by a CCD detector (Princeton Instruments, Pixis 100). The spectrometer is calibrated as follows: a mercury argon calibration source is utilized in the calibration and the

front slit of monochromator is set to 20 μm . Then the light from calibration source is aligned into the monochromator via the front slit and the spectral intensity with pixel index is recorded. Finally, the relationship between pixel index and wavelength is fitted by a 2-order polynomial equation, as shown in Figure 3.8. With the calibration, the error of wavelength is less than 0.3 nm in our setup.

3.4.3 Femtosecond transient absorption spectrometer

Figure 3.9: Schematic representation of femtosecond transient absorption spectrometer. Wd: Wedge Window; L1: Lens, $f = 75\text{mm}$; Chp1 and Chp2: Optical Chopper; Pol1 and Pol2: Polarizer; PM1 and PM2: Parabolic Mirror, $f = 6$ inches. AL1 and AL2: Achromatic lens, $f = 50$ mm, 100 mm.

The setup of our new femtosecond transient absorption spectrometer is shown in Figure 3.9. In our setup, two polarizers are utilized. The second polarizer Pol2 is fixed at the magic angle 54.7° while the first polarizer Pol1 is used to adjust the power of the pump beam. Additionally, there are scattered light generated due to the large particle sizes of the photosynthetic. In order to remove the effect of the scattering light and the CCD thermal noise, two optical choppers are used in the setup. The chopper Chp1 is placed in the path of pump light and set to 250 Hz. The second chopper, placed in the path of the probe beam, is set to 500 Hz. In this case groups of four shots are collected in a loop to calculate the absorption difference spectrum:

$$\Delta A(\lambda) = -\log \left(\frac{I(\lambda)_{\text{pumped}+\text{sca}+\text{bn}} - I(\lambda)_{\text{sca}+\text{bn}}}{I(\lambda)_{\text{unpumped}+\text{bn}} - I(\lambda)_{\text{bn}}} \right) \quad (3.1)$$

where $I(\lambda)_{pumped+sca+bn}$ is the signal when both of the pump and probe beams are unblocked, $I(\lambda)_{sca+bn}$ is the signal while only the pump beam is unblocked, $I(\lambda)_{unpumped+bn}$ denotes the signal when only the probe beam is unblocked and $I(\lambda)_{bn}$ is signal in the case when both of the pump and probe beams are blocked.

3.5 Data Analysis

The spectroscopic signals which are obtained by pump probe spectrometer are a superposition of the characteristic of the different spectral components weighted by their coefficient and the absorption difference spectra at different wavelengths obey the Beer-Lambert law [3.29]. Thus, the related equation can be written as:

$$\psi(\lambda, t) = \sum_{n=1}^N c_n(t) \exp(-t/\tau_n) \phi_n(\lambda) \quad (3.2)$$

where $c_n(t)$ denotes the coefficient and is termed a kinetic model, $\phi_n(\lambda)$ denote the spectrum and is termed a spectral model. Transient absorption data are analysed by using a kinetic model consisting of decay-associated difference spectra (DADS), indicate successive mono-exponential decays of increasing time constants, which can be regarded as the lifetime of each DADS. This method usually is called as global analysis. From the equation, we can see the results are two-way data, and the first way is the experimental spectral variable (wavelength λ) directly recorded from CCD detector and the second way is the experimental variable which is used to record spectral change: time t after excitation.

In our ultrafast transient absorption spectroscopy, the molecular system is excited by a short laser pulse of certain energy and probed by a white-light continuum. The convolution of the shapes of the pump pulse and the probe pulse is called the instrument response function (IRF) and this IRF is an important parameter when the data is analysed. On a short time scale (ps or fs or as), the shape of IRF represents the timing precision. However, the IRF is also affected

by the solution including solvent and sample, the length of the sample cell and so on. The IRF may be different in the different experiments [3.30].

Figure 3.10: Schematic representation of measurement of Kerr effect. PM1 and PM2: parabolic mirrors; Pol1 and Pol2: polarizer.

Figure 3.11: The measurement of Kerr effect and Gaussian Fit.

One common way to measure the IRF is utilizing the Kerr effect of the solution [3.31]. The measurement setup of the Kerr effect is shown in Figure 3.10. The first polarizer Pol1 is set

to 0° and the second one is set to 90° , the exposure time of CCD is set to a long time (more than 1s). Only when the pump beam is unblocked, a small fraction of light will be detected by the CCD. Then the spectra are collected in wavelength at the different delay time: $A(\lambda, t) = \Lambda(\lambda)K(t)$. The function $K(t)$ can be considered as the IRF at certain wavelength λ , as illustrated in Figure 3.11.

The data of the difference absorption spectra is analysed with a sufficient number of exponential decays associated with their amplitudes $c_n(t)$ termed decay associated difference spectra (DADS). The $c_n(t)$ can be described by convolution of multi-exponential decay with the IRF [3.29], as follows:

$$c_n(t) = \sum_i \exp(-k_i t) * K(t)$$

$$= \sum_i \exp(-k_i t) * \sqrt{\frac{2 \ln(2)}{\pi}} (-4 \ln(2)) ((t - t_0)/\sigma)^2 \quad (3.3)$$

Figure 3.12: The results of pump probe measurements. Dash line: the polynomial fit of the wavelength-dependent time delay of the continuum.

As mentioned in Chapter 3.4.1, a white-light continuum is used as the probe beam in the pump probe experiment. It is known that self-phase modulation (SPM) is the primary process

of the continuum generation. The SPM rarely has an effect on the temporal width, but causes an upchirp in the continuum pulses [3.32]. During the transmission of the continuum, air and lenses add extra upchirp in the time domain as well. The chirp of the continuum causes that different wavelengths components reach the sample at the different delay time. In order to obtain the correct dynamics, we will deal with the chirp in the process of data analysis. The coherent artifact between pump and probe beams only happen at around time zero, so we can get the wavelength-dependent time delay of the continuum by tracking positive central peaks of the artifact signal in the results of pump probe measurements. The wavelength-dependent temporal structure can be good fitted by a 2-order polynomial equation [3.32, 3.33], as shown in Figure 3.12. This equation will be used to calculate the real time zero at different wavelength in the analysis process.

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Chapter 4

Experimental Study of Energy Transfer from the Soret Band to Q_y Band in Chlorophylls

4.1 Introduction

Chlorophylls are magnesium-containing substituted tetrapyrroles and play a vital role in the light harvesting machinery of green plants [4.1, 4.2]. In photosynthesis, chlorophylls absorb the photons and transfer the light energy to other parts of the photosystems [4.3].

Due to the importance of chlorophylls, a number of relative researches about chlorophylls have been performed by different groups. In 1979, the triplet and excited singlet absorption spectra of chlorophyll *a* in pyridine have been investigated by Baugher's group. They found the excited triplet state spectrum is broad spectrum with three peaks at 460 nm, 380 nm and 540 nm. The excited singlet spectrum has a peak at 440 nm with a spectral width of about 60 nm. No absorption above 500 nm in wavelength is found, which is quite different with ground state absorption [4.4]. Lokstein et al have carried out an interesting experiment to study excitationally coupled chlorophylls *a* and *b* in light harvesting complex II which are isolated from peak leaves. They use the laser pulses to pump the first excited singlet state Q_y band and probe the Soret region of chlorophyll *a* and *b*. Through this experiment, they found some chlorophyll *a* which absorb at 678 nm is strongly excitationally coupled to chlorophyll *b* [4.5]. Recently, Kobayashi's group use a few-cycle pulse laser with temporal width of 6.8

fs to obtain both electronic relaxation and vibrational dynamics of the Q_y band of chlorophyll *a* in room temperature. They observe more than 25 vibrational modes are coupled to the Q_y band. They also observe the excited state vibrational coherences [4.6].

Moreover, from the previous researches [4.7, 4.8, 4.9], we know that the first singlet excited state S_1 of chlorophyll corresponds to the Q_y absorption band, and the Q_x absorption band is from the second excited singlet state S_2 and the upper singlet states S_n correspond to the Soret absorption band which also is named B band. Generally, between Q_x and Q_y bands, another absorption band can be found and this absorption band is shown to be the first vibronic replica of the Q_y origin band and assigned as Q_{y1} band.

In this chapter, a pump beam at 442 nm was used to excite the Soret band and a broad band beam of white-light continuum was used to probe the Q_x , Q_y and Q_{y1} bands in transient absorption spectroscopy to obtain the femtosecond relaxation dynamics from B band to Q band in chlorophyll *a* and *b*. Part of results have been published in [4.11].

4.2 Material and Experimental Method

4.2.1 Femtosecond Transient Absorption Spectrometer

Our transient absorption spectrometer has been described in Chapter 3.4 and in this experiment the pump laser was adjusted to 442 nm and with pulse energy of 100 nJ with a pulse duration of 55 fs as measured by a home-built autocorrelator. The measured pulse width is also corroborated by the recorded lifetime of coherent artifact of the solvent. The pump and probe pulses were focused to a 100 μ m diameter spot and spatially overlapped in the sample with a 90° off-axis parabolic mirror with reflected focal length 6 inches. The polarization of the pump beam was set to the magic angle of 54.7° in order to obtain the population-only dynamics. Absorption difference spectra were recorded at pump-probe

delays between -1 and 8 ps with a minimum step of 10 fs. Steady-state absorption spectra were taken before and after the transient absorption measurements to check for the integrity of the sample. All measurements were performed in the dark at room temperature (around 25°C).

4.2.2 Sample Preparation

The powder samples were purchased from Sigma-Aldrich and then dissolved in HPLC grade ethanol alcohol from the same company. We directly used all the chemicals without further purification. The solutions were put into a 2 mm path length cell and adjusted to provide an optical density of 0.8 at the Soret band at 442 nm.

4.3 Results and Discussion

4.3.1 Ground State Absorption Spectra

Figure 4.1: Ground State Absorption Spectra of Chlorophyll *a* and *b* in ethanol and pump spectrum.

The ground state absorption spectra of chlorophyll *a* and *b* in ethanol at room temperature have been measured, as shown in Figure 4.1. According to the absorption spectra, for chlorophyll *a*, the lowest-energy Q_y electronic absorption band is located at 664 nm and the vibronic replica Q_{y1} band is located at 618 nm, the center wavelength of the second excited singlet state S_2 band is 588 nm, and the Soret band is from 400 nm to 450 nm. The absorption spectra of chlorophylls result from the linear combination of one-electron promotions, and the $\pi\pi^*$ transitions are responsible for the two strong absorption bands: Soret and Q bands. The Soret band comprises some upper singlet states due to the spin state forms and so on [4.12, 4.13]. Comparing with chlorophyll *a*, all the Q bands of chlorophyll *b* are blue shifted. The Q_y absorption band of chlorophyll *b* is shifted to 649 nm, the Q_x band is at 558 nm, and the Q_{y1} band is at 600 nm. However, the Soret band of chlorophyll *b* is red shifted and the range is from 400 nm to 500 nm. Also, it is very clear that the gap between Soret band and Q_y band of chlorophyll *a* is much bigger than the gap between B band and Q_y band of chlorophyll *b*.

4.3.2 Transient Absorption Spectra of Chlorophylls

Figure 4.2: Transient absorption spectra of chlorophyll *a* in ethanol. Delay time between pump and probe beams is from -1 to 8 ps.

To investigate the energy transfer process from the Soret band to the Q_y band in chlorophylls, time-resolved absorption spectra in chlorophyll *a* and *b* at room temperature were measured upon excitation with 55 fs pulses at 442 nm corresponding to the Soret bands of chlorophylls and all the effects of the chirped white light continuum on the results were corrected (see Section 3.5 for details).

The transient absorption spectra of chlorophyll *a* in a spectral range from 580 nm to 720 nm are shown in Figure 4.2, plotted against the delay time between pump and probe pulses (from -1 to 8 ps). In this wavelength region, the Q_y absorption band of chlorophyll *a* dominates the absorption spectrum. A large negative signal with a maximum at around 670 nm dominates the total transient absorption spectra and is due to ground state bleaching and stimulated emission.

Figure 4.3: Transient absorption spectra of chlorophyll *b* in ethanol. Delay time is from -1 to 8 ps.

Under the Same parameters, the transient absorption spectra of chlorophyll *b* are represented in Figure 4.3. The absorption spectra are dominated by a negative band with a maximum at around 652 nm from the transition of the Q_y absorption band of chlorophyll *b*.

Figure 4.4 shows the transient absorption profiles of chlorophyll *a* and *b* for two different time delays 0.5 and 8 ps. At both delays of 0.5 ps and 8 ps, the transient absorption spectra of chlorophyll *a* and *b* exhibit maximum negative peaks at 670 nm and 652 nm, respectively. The wavelengths of the maximum negative signals due to bleaching and stimulated emission have no observable shifts from time delay of 0.5 ps to 8 ps. The characterization of wavelength features of chlorophylls is almost in agreement with the absorption bands of ground state absorption spectra shown in Figure 4.1. This increase of the amplitudes from 0.5 ps to 8 ps of the peaks at 670 nm and 652 nm for chlorophyll *a* and *b*, respectively, indicates the excitation energy is being transferred to the strongest Q_y absorption bands of chlorophylls.

Figure 4.4: Transient absorption spectra at 0.5 and 8 ps. (a) chlorophyll *a*; (b) chlorophyll *b*.

For chlorophyll *a*, as shown in Figure 4.4 (a), a positive signal from 580 to 650 nm is found in transient absorption spectra and includes two peaks at 640 and 590 nm. The amplitude of the 640 nm positive peaks within delay time of 8 ps. This increase is correlated with the dynamics of the negative peak at 672 nm, and can be attributed to excitation-state absorption. On the other hand, the signal at 590 nm remains unchanged. This may be because of the combined action of the excited absorption and the transition of the Q_x absorption band which is located at 588 nm. For chlorophyll *b*, a positive signal can be observed from 580 to 640 nm

and this signal also is due to the excited state absorption of the Q_y absorption band. The amplitude of the positive absorption difference spectra of chlorophyll *b* also increases slightly at 8 ps, this is because the excitation energy is transferred to the S_1 state corresponding to the Q_y absorption band.

4.3.3 Transient Absorption Kinetics Traces

Figure 4.5: Representative pump pulse induced transient absorption kinetics curves for chlorophyll *a* exciting with 55 fs pulses at 442 nm and the transient absorption at 640, 670 and 680 nm.

The pump pulse induced absorption kinetics traces for chlorophyll *a* recorded at three different wavelengths (640, 670 and 680 nm) are shown in Figure 4.5. All the kinetics traces have similar features. In the first few hundred femtoseconds, a fast rise of the total signal occurs. The total signal is well fitted with two time constants of 126 fs and 5 ns.

In order to obtain the detailed information on the different spectral components which contributes to the transient absorption kinetics at different wavelengths, the collected data was analysed by global analysis techniques [4.10] (as described in Section 3.5). The global analysis was performed for transient absorption spectra from -1 ps to 8 ps in the range of 580-

720 nm. For both of chlorophyll *a* and *b*, a two-component kinetic scheme was used in the global analysis with a fast component representing the transition times from the Soret band to the Q_y band and one quasi-stationary component representing the lifetime of the S_1 state. In this chapter, our purpose is to study the energy transfer dynamics between the S_n state and the S_1 state, this process is only around a few picoseconds. In our measurements, the maximum time delay is up to 8 ps. Generally, the lifetimes of the S_1 states of chlorophyll *a* and *b* are much longer than the studied process and do not have an effect on the energy transfer dynamics between the Soret and Q_y bands. Thus a long time component which is fixed to 5 ns is used to represent the lifetime of the S_1 state of chlorophyll in our analysis process. The transition times between the Soret band and the Q_y band, and the decay associated difference spectra (DADS) are obtained by the fit.

Figure 4.6: Chlorophyll *a* excited at 442 nm at room temperature. Decay associated difference spectra from measured data in the region of Q_y absorption band.

Figure 4.7: Representative pump pulse induced transient absorption kinetics curves for chlorophyll *b* exciting with 55 fs pulses at 442 nm and recording at 670, 652 and 620 nm.

The DADS from the global analysis for chlorophyll *a* is illustrated in Figure 4.6. The first DADS (black curve), corresponding to the lifetime of 126 fs, shows a fast gain of absorption of the S_1 state. The positive signal of DADS in the spectral range from 645 nm to 700 nm represents the absorption difference signal due to bleaching and stimulated emission of the Q_y absorption band rapidly rise while the negative signal of DADS below 645 nm means that the excited state absorption signal also rapidly increase.

Figure 4.8: Chlorophyll *b* excited at 442 nm at room temperature. DADS from collected data in the region of the Q_y band.

For chlorophyll *b*, the absorption kinetics which is induced by the pump pulse with probe wavelengths at three different wavelengths (670, 652 and 620 nm) are illustrated in Figure 4.7. Similar to the kinetics traces of chlorophyll *a*, all the kinetics traces of chlorophyll *b* have similar features including a fast rise in the first hundreds femtoseconds. The rise of transient absorption signal consists of a fast rise with time constant of 156 fs. The time constants of the fast rise for chlorophyll *b* are slightly larger than for chlorophyll *a*. This implies the energy transfer rate between the Soret band to the Q_y band in chlorophyll *a* faster than chlorophyll *b* although the energy gap of chlorophyll *a* is bigger than chlorophyll *b*.

Figure 4.6 shows the DADS obtained by the global analysis for chlorophyll *b*. The DADS is modelled using three components and the fit of the lifetimes are: 156 fs, and 5 ns. The first

component of DADS (black) with the lifetime of 156 fs shows a fast gain of absorption of the Q_y band. The transient absorption signal due to photobleaching and stimulated emission in the spectral range of 635-700 nm rapidly increases while the signal of DADS is positive. The positive absorption difference spectra due to excited state absorption also fast rise because of the negative signal of DADS below 640. The last DADS (blue) is attributed the decrease of transient absorption spectra, and at 5 ns most of chlorophyll *b* molecules have been back to the ground state.

4.3.4 Discussion and Analysis

Transient absorption spectra upon excitation with 55 fs pulses at 442 nm in chlorophyll *a* and *b* were investigated at room temperature. The absorption difference spectra from 580 to 720 nm were collected as a function of the time delay between pump and probe pulses corresponding to the Soret bands of chlorophyll *a* and *b*.

The results of the measurements in the spectral range of 580-720 nm are summarized. A negative band due to photobleaching and stimulated emission and a positive band due to excited state absorption dominate the transient absorption signal. In transient absorption spectra of chlorophyll *a* and *b*, compared to the absorption bands of ground state absorption of chlorophyll *a* and *b*, the maximum negative peaks at 670 nm and 652 nm are exhibited which is induced by a dynamics red-shift of the maximum bleach of the pump pulse. This dynamics red-shift is because of the composition of the bleaching signal and the stimulated emission signal. Typical transient absorption kinetics traces at 670 nm and 652 nm for chlorophyll *a* and *b* is represented in Figure 4.9. Both the kinetics curves show a rapid increase followed a slower phase in the signals. It is evident that the energy transfer dynamics in chlorophyll *a* and *b* is very similar. The rapid rise represents the transient absorption difference signal due to ground-state photobleaching while the slower rise is possibly due to

stimulation emission of the first singlet S_1 state. The slower rise of the absorption different spectra indicates the internal conversion from the S_n state to the S_1 state [4.11].

Figure 4.9: Typical transient kinetics traces at 670 nm for chlorophyll *a* and 652 nm for chlorophyll *b*.
The signal of chlorophyll *b* are multiplied by 2.36.

For both of chlorophyll *a* and *b*, upon the excitation of the Soret band with 55 fs pulses at 442 nm the molecules are pumped to the excited singlet S_n state. Therefore, the excitation energy transfer processes $Soret \rightarrow Q_y$ are described in the transient absorption spectra. Three lifetimes in the global analysis were used to fit the experimental data with the third lifetime of 5 ns. The two shorter lifetimes of 114 fs and 5.32 ps in chlorophyll *a* have negative amplitudes in the spectral range of 580 to 645 nm and positive amplitudes in the range of above 645 nm. Because the transient absorption signal in the range of 580- 645 nm is positive and a negative signal above 645 nm is shown, both lifetimes are rise times in the transient absorption kinetics. The rise times represent the photobleaching of ground-state and excitation energy transfer from the Soret band to the Q_y band, respectively. In chlorophyll *b*, the two shorter lifetimes are changed to 149 fs and 6.4 ps with negative amplitudes in the spectral range of

580 to 635 nm and positive amplitudes in the range of above 635 nm. Same reason with chlorophyll *a*, both time constants illustrates the rise times which represent the ground-state bleaching and excitation energy transfer from the S_n state to the S_1 state. The shorter lifetime of 149 fs also dominates the photobleaching signal and the larger time constant of 6.4 ps illustrates the processes of internal conversion between S_n and S_1 states. No significant effects of the Q_x band and Q_{y1} band are observed in our experiment.

The energy gaps between the Soret band and the Q_y band of chlorophyll *a* and *b* are around 7699 cm^{-1} and 7287 cm^{-1} , respectively. However, for the analysis of the experimental data, the time constant of internal conversion between the S_n and S_1 states in chlorophyll *b* is slightly larger than that in chlorophyll *a*. In summary, the energy transfer process between the Soret and Q_y bands of chlorophyll *a* is slightly faster than that of chlorophyll *b*. But, it is to be noted that the differences between the lifetimes of the energy transfer dynamics between the Soret band and Q_y bands in chlorophyll *a* and *b* are very close to or within the error estimates in the experiment, and we did not find any other results about the energy transfer rate between the Soret and Q_y bands in chlorophyll.

To verify the energy transfer dynamical processes in chlorophyll *a* and *b*, the non-adiabatic excited-state molecular dynamics method has been employed to study the internal conversion processes in chlorophyll *a* and *b* [4.11]. In the calculation, the geometry of each chlorophyll molecule was optimized by using Gaussian 09 [4.14], and the ground and excited state dynamics was simulated at the AM1/CIS level of theory [4.15, 4.16]. Through the calculation, the energy gap between Soret and Q_y bands in chlorophyll *a* is 1.24 eV, with the energy gap in chlorophyll *b* is 1.12 eV. The calculated results are in reasonable agreement with the experimental results (1.01 eV in chlorophyll *a*, 0.76 eV in chlorophyll *b*). These calculated results follow the trends of the experimental results, where the energy gap between Soret and Q_y bands in chlorophyll *a* is bigger than the gap in chlorophyll *b*.

Figure 4.10: The excited state population of Q_y , Q_x and Soret bands in chlorophyll *a* and *b* as a function of time delay [4.11].

The population evolutions of Soret and Q_y bands in each chlorophyll molecule are calculated by the NA-ESMD method. In the calculation, the excited states from S_3 to S_{10} state comprised the Soret band, and the Q_y band is the S_1 state. The Q_x band (S_2 state) also is considered into the simulation. The calculated results and the fit curves for the population of Q_y , Q_x and Soret bands are shown in Figure 4.10. The fit curves were obtained by a least-squares regression analysis and the time constants are presented in Table 2. From Table 2, we can see that the lifetime for energy transfer dynamics from Soret $\rightarrow$ Q_y in chlorophyll *a* (227 fs) is slightly smaller than the lifetime in chlorophyll *b* (267 fs); this conclusion is well in agreement with experiment. The calculated results also show the time constants from

Soret $\rightarrow$ Q_x and Q_x $\rightarrow$ Q_y processes, but the two pathways were not resolved in our experiment because of the probe range.

	Soret $\rightarrow$ Q _x	Q _x $\rightarrow$ Q _y	Soret $\rightarrow$ Q _y
	τ_1 (fs)	τ_2 (fs)	τ_{total} (fs)
Chlorophyll <i>a</i>	99.2 $\pm$ 1.1	128 $\pm$ 2	227 $\pm$ 3
Chlorophyll <i>b</i>	57.9 $\pm$ 1.4	208 $\pm$ 3	267 $\pm$ 4

Table 2: The time constants obtained from the first-order irreversible model fits for Q_y, Q_x and Soret bands.

4.4 Conclusion

In this chapter, we have discussed the energy transfer dynamics between the Soret and Q_y bands of chlorophyll *a* and *b*. The dynamical processes of chlorophyll *a* and *b* are shown in the transient absorption spectra measured at room temperature. These red-shifts have been assigned to the composition of the bleaching signal and the stimulated emission signal. At delay times of few hundreds of femtoseconds and few picoseconds, the transient absorption signal exhibits the photobleaching and stimulated emission at longer wavelength and the excited state absorption at shorter wavelength. Global analysis of the experimental data indicates the excitation energy transfer between the Soret and Q_y bands in chlorophyll *a* and *b* occurs on subpicoseconds and picoseconds. Lifetime of 126 fs illustrates the S_n $\rightarrow$ S₁ internal conversion processes in chlorophyll *a*, while in chlorophyll *b* the two lifetimes are measured to be 156 fs. Although the energy gap between the Soret and Q_y bands in chlorophyll *b* is smaller than that in chlorophyll *a*, the energy transfer process in chlorophyll *b* is slower than that in chlorophyll *a*. The simulation results also support that the internal conversion from Soret $\rightarrow$ Q_y in chlorophyll *a* is slightly faster than that in chlorophyll *b*.

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Chapter 5

Ultrafast Pump Probe Measurements of Carotenoid to Chlorophyll Energy Transfer in Different Light-Harvesting Complex II

5.1 Introduction

As the most abundant light-harvesting antenna pigment-protein complex associated with photosystem II in higher plant, the light-harvesting complex II (LHC II) plays an important role in the absorption of light and efficient transfer of electrons and excited state energy in photosynthetic reactions [5.1, 5.2]. The large majority of all chlorophylls on earth are bound in LHC IIs [5.3]. In general, LHC II exists as a trimeric unit, which is mainly composed of three monomeric products resulting from expression of the genes of *Lhcb1*, *Lhcb2* and *Lhcb3* [5.4] and organized as three transmembrane α -helices (helices A, B and C). Each monomeric LHC II binds 7-8 chlorophyll *a* molecules, 5-6 chlorophyll *b* molecules and 3-4 carotenoids [5.5]. In addition, the structural features of LHC II also include a short amphiphilic α -helix (helix D) and one amphipathic 3_{10} -helix (helix E) [5.6].

The crystal structure of major LHC II from spinach has been reported at the resolution of 2.72 Å by means of the X-ray crystallography [5.6]. The resolution is unambiguous in distinguishing the 14 chlorophylls as 8 chlorophyll *a* molecules and 6 chlorophyll *b* molecules and to observe four carotenoid-binding sites per monomeric LHC II, and the orientation of the lowest-energy electronic transition dipole moment of each chlorophyll has

been assigned. The carotenoids associated with LHC II have an important and special function which is the prevention of the photosynthetic systems from damage through the quenching of chlorophyll triplet states which can form damaging singlet oxygen by reacting with oxygen [5.7]. Carotenoids also absorb the light in the blue green spectral region which complements the chlorophyll molecules absorption in the red spectral region. The excitation energy absorbed by carotenoids can be transferred to the chlorophylls [5.8]. In carotenoids, the absorption from the ground state S_0 ($1^1A_g^-$) to the first excited singlet state S_1 ($2^1A_g^-$) is forbidden by the selection rule due to the C_{2h} symmetry of both states while the strong absorption bands in the visible spectral region are due to the transition from the ground state to the second excited state S_2 ($1^1B_u^+$) [5.9]. Generally, the lifetime of the S_1 state is tens of picoseconds and the lifetime of the S_2 state is hundreds of femtoseconds [5.9, 5.10, 5.11]. These spectral properties of carotenoids, such as rapid internal conversion, short lifetimes of excited states and low coherence between the ground state and first excited state, provide efficient quenching mechanism and the carotenoid to chlorophyll energy transfer dynamical processes [5.11].

The energy transfer dynamics in LHC II has attracted the interest of many groups already and some general conclusions about energy transfer among chlorophylls have been obtained. The lifetimes of energy transfer from chlorophyll *b* to chlorophyll *a* consist of three components: 150-200 fs, 500-600 fs and 5-7 ps while the energy transfer among chlorophyll *a* molecules occurs over a time period longer than 1.0 ps [5.12, 5.13]. In order to study the important structural and functional properties of LHC II, the molecular genetics and reconstitution techniques have also been applied to the spectroscopic research. The genetic and reconstitution technique can be used to exchange the pigments in the LHC II with different pigments, and through this tool the energy transfer pathways will be further characterized. Yang et al have made three recombinant LHC IIs (V119F, H120L and S123G) with the three

amino acids (Val-119, His-120 and Ser-123) in an antiparallel strands between helices B and C structure replaced by phenylalanine, leucine and glycine respectively. The measurements of circular dichroism spectra and low temperature fluorescence emission spectra were carried out and they found that this antiparallel strands have a big impact on their fluorescence spectra [5.14]. Some measurements about the carotenoid to chlorophyll energy transfer have also been reported. The femtosecond transient absorption spectroscopic measurements were applied to two recombinant LHC II (LHCII-lutein and LHCII-neolutein) by Holzwarth's group and they found the energy transfer from carotenoid to chlorophyll occurs from the second excited state S_2 with time constants of less than 100 fs, and half of excitation energy absorbed by carotenoids is transferred to chlorophyll *b* while chlorophyll *a* directly accept the rest energy [5.3].

As mentioned in the upper paragraph, the genetic technique can be used to change the structure of LHC II, and then many different types of LHC II have been produced. Through this reconstitution tool, the amino acid His-120 in the gene of *Lhcb1* was changed to leucine, and then the H120L mutant LHC II will be obtained. One clear characteristic of this mutant LHC II is the different antiparallel strands between helices B and C. In order to clearly clarify the effect of His-120, the wild type LHC II, which is expression of the entire LHC II genes without any changes obtained from higher plant in the cyanobacterial, is also studied. Furthermore, the reconstitution in the cyanobacterial also has possible effects on the energy transfer dynamics. The native LHC II directly isolated from the pea leaves is used as control group and to describe the effects. In this chapter, femtosecond transient absorption spectroscopic measurements will be performed and applied to three types of LHC IIs (native, wild type and H120L) to study the effects of the antiparallel strands on carotenoid to chlorophyll energy transfer process, and native and wild type LHC IIs will be studied as control group.

5.2 Materials and Methods

Native LHC II is directly isolated from the leaf of 14-day-old pea, and the wild type LHC II is the product resulting from the expression of the genes of higher plant growing in cyanobacterial. The H120L mutant LHC II is obtained by using the same method with Yang's group [5.14].

The femtosecond pump-probe spectrometer used in this experiment has been described in Chapter 3.4. In this experiment, the pump beam was set to 500 nm, and the pump pulse energy was adjusted to 5 nJ. A home-built autocorrelator was used to check the pulse duration, and the temporal width of the pump pulses is around 50 fs. The diameter of focus point was calculated to be 100 μm . To obtain the population-only dynamics, the polarization of the pump beam was set to the magic angle of 54.7° . Linear ground state absorption spectra were taken before and after the transient absorption measurements to check for the integrity of the sample. All the pump probe measurements were performed at room temperature (around 25°C).

5.3 Results

5.3.1 Transient Absorption Difference Spectra

Femtosecond transient absorption spectra of native, wild type and H120L mutant LHC II were measured upon excitation with 50 fs pulses at 500 nm corresponding to the absorption of carotenoid at room temperature. In each case, the absorption difference spectra in a spectral range from 570 nm to 700 nm were taken within the time delays of -2 to 800 ps. The white-light continuum, generated by focusing ultrashort 800nm pulse into a sapphire window, is used as the probe light in our experiment. The wavelength-dependent temporal structure of the probe light is shown in the pump probe measurements by the coherent artifact between

the pump and probe lights. A second-order polynomial equation (wavelengths as a function of time delay) is utilized to correct the dispersion effect. The detail has been described in the Section 3.5. The transient absorption kinetics were analysed by a global fitting analysis and the number of components for analysis was determined by the residual.

Absorption difference spectra in the chlorophyll absorption spectral region for native LHC II are illustrated in the left part of Figure 5.1. A large negative signal with a maximum at around 682 nm dominates the total transient absorption spectra while the positive signal is found in the higher frequency region, indicating all the excitation energy is transferred to the 682 nm chlorophyll *a* finally.

Figure 5.1: (Left): The contour map of transient absorption spectra for native LHC II as a function of time delay and wavelength. (Right): Transient absorption profiles for native LHC II at the time delays: 100 fs, 1 ps, 10 ps and 100 ps.

The right part of Figure 5.1 shows the transient absorption profiles for native LHC II at four different delay times of 100 fs, 1 ps, 10 ps and 100 ps between the pump and probe pulses, and some details of the dynamical processes can be directly obtained from it. At 100 fs after the pump pulse, the transient absorption spectrum exhibit three broad negative peaks. In the spectrum, the peak at 682 nm corresponds to the Q_y absorption band of bounded chlorophyll *a*, a shoulder at 673 nm is due to the absorption of unbound chlorophyll *a* [5.5]. The peak at

654 nm results from the Q_y band of chlorophyll *b*. The small broad negative peak at around 605 nm should be due to the Q_x band of chlorophyll *a*. These results are similar with the report in [5.13, 5.14]. At the delay time of 1.0 ps, the negative signal at around 654 nm decreases with an increase of the strength of the signals at around 682 nm and 673 nm, and these indicate the energy transfer from chlorophyll *b* to chlorophyll *a*. The broad negative peak at around 605 nm disappears, meaning the internal conversion process of chlorophyll *a*. The positive signal in the shorter wavelength region (570-640 nm) due to the excited state absorption of native LHC II appears. Furthermore, at 10 ps after the excitation pulse, the bleaching and stimulated emission signal at around 654 nm also disappears and the negative signal near 682 nm rises. These mean almost all the energy has been transferred to chlorophyll *a*. At 100 ps after the pump pulse, only the decreasing signal near 682 nm exists, and the shoulder at around 673 nm also disappear, indicating that the population of native LHC II is being transferred to the ground state and the excitation energy is transferred from the unbound chlorophyll *a* to the bound chlorophyll *a* [5.5, 5.11].

For wild type LHC II, the experiment data is illustrated in the left part of Figure 5.2. Similar with native LHC II, the bleaching and stimulated emission around 681 nm are dominant in the total absorption difference spectra, and the positive signal from 570 nm to 640 nm due to the excited state absorption exists at the shorter wavelengths.

Figure 5.2: Left part: The contour map of the raw experimental data for wild type LHC II as a function of time delay and wavelength. Right part: Transient absorption spectrum for wild type LHC II at the time delays: 100 fs, 1 ps, 10 ps and 100 ps.

The transient absorption spectra of wild type LHC II at four different delay times of 100 fs, 1 ps, 10 ps and 100 ps after the excitation pulse are shown in the right part of Figure 5.2. A part of the dynamical processes of wild type LHC II are directly visible from the raw absorption spectra. At the delay time of 100 fs, three broad negative peaks at around 605 nm, 652 nm and 681 nm corresponding to the S_2 state of chlorophyll *a*, the S_1 state of chlorophyll *b* and the S_1 state of chlorophyll *a* respectively are exhibited in the absorption difference spectrum. A shoulder at 672 nm of the 681 nm peak is also found in the spectrum, and it is due to the unbound chlorophyll *a*. The bleaching and stimulated emission signals of the Q_y bands of chlorophyll *a* and chlorophyll *b* are slightly blue shifted compared to the signal from the native LHC II. At 1.0 ps after the pump pulse, the negative signal at around 652 nm decreases with an increase of the signal of bleaching and stimulated emission of the S_1 states of chlorophyll *a* and unbound chlorophyll *a*, and these illustrate the excitation energy is transferred from chlorophyll *b* to chlorophyll *a* and unbound chlorophyll *a*. The broad peak at around 605 nm disappears, which indicates the $S_2 \rightarrow S_1$ energy transfer process in chlorophyll *a*. In the spectral range of 570-640 nm the positive signal due to the excited state absorption of wild type LHC II is found. At the delay time 10 ps between the pump and probe pulses, the

bleaching and stimulated emission signal at 652 nm disappear with an increase of the negative signal near 681 nm, and these mean that almost all the excitation energy of chlorophyll *b* has been transferred to chlorophyll *a*. The 100 ps spectrum lacks a shoulder at around 672 nm, indicating that there is energy transfer process from the unbound chlorophyll *a* to chlorophyll *a*.

Figure 5.3: Left part: The contour map of the collected data for H120L mutant LHC II as a function of time delay and wavelength. Right part: Transient absorption spectrum for the H120L mutant LHC II at the time delays: 100 fs, 1 ps, 10 ps and 100 ps.

With the same scales used in native and wild type LHC II, the absorption difference spectra of the H120L mutant LHC II obtained by femtosecond transient absorption spectrometer are presented in Figure 5.3. At the delay time of 100 fs, a broad negative peak at around 681 nm with two shoulders at around 673 nm and 651 nm and a small broad negative peak at around 605 nm are shown in the spectrum. The 681 nm peak is due to the Q_y band of chlorophyll *a*, the 673 nm shoulder results from the unbound chlorophyll *a*, and the 651 nm shoulders is because of the bleaching and stimulated emission of chlorophyll *b*. Compared with native and wild type LHC II, the relative intensity of the signal around 651 nm is quite small, which indicates that most of the excitation energy is transferred from carotenoids to chlorophyll *a* directly and with less energy being transferred via chlorophyll *b*. At the delay time of 1 ps,

the signal of bleaching and stimulated emission of chlorophyll *b* at around 650 nm and the peak at around 605 nm due to the Q_x band of chlorophyll *a* disappear, which means that almost all the energy has been transferred from chlorophyll *b* to the Q_y band of chlorophyll *a* and the $Q_x \rightarrow Q_y$ internal conversion process of chlorophyll *a* occurs, respectively. When the delay time is 10 ps, only the unbound chlorophyll *a* to chlorophyll *a* energy transfer occurs. The excited H120L mutant LHC II decays to the ground state at 100 ps.

5.3.2 Global Analysis

The experimental spectra were analysed by the global analysis to gain the detailed information on the transient absorption kinetics profiles at different wavelengths. The global analysis with five necessary decay components was performed for the measured absorption spectra from -2 to 10 ps in the range of 620-720 nm with the least residual. DADS for three samples excited at 500 nm are obtained.

Figure 5.4: Time-resolved absorption difference profiles for native LHC II pumped at 500 nm and probe at 682 nm, 673 nm and 654 nm. Scatter: raw data; Solid curve: the fit.

For native LHC II, transient absorption difference kinetics curves at the special wavelengths (654 nm, 673 nm, 682 nm) in the Q_y region of chlorophyll are shown in Figure 5.4. At probe wavelength 654 nm in the Q_y band of chlorophyll *b*, a fast rise exists in the kinetics curve on

a time scale of hundreds of femtoseconds and the signal decays on few of picoseconds. However, a fast rise with slower phases appears in the kinetics curves at the probe wavelengths 673 nm and 682 nm, after 5 ps, the 682 nm signal is still slowly rising while the 673 nm peak already starts to slowly decrease, and these processes of two wavelengths describe the dynamical processes of the Q_y absorption bands of chlorophyll *a* molecules.

Figure 5.5: DADS resulting from global analysis of the measured data upon excitation with 50 fs pulses at 500 nm for native LHC II.

A global analysis was applied to analyse the transient absorption spectra for native LHC II, and five time constants were used to in the analysis: 139 fs, 817 fs, 7.71 ps, 69.1 ps, and 1.41 ns. The kinetics traces get a satisfactory fit as shown in Figure 5.4. Because the maximum delay time of absorption spectra for native LHC II is 800 ps, the longest-living component 1.41 ns is not very accurate. Figure 5.5 shows the DADS resulting from the global analysis. The longest-living 1.41 ns DADS represent the bleaching and stimulated emission signals due to the Q_y band of chlorophyll *a*. The 139 fs DADS clearly shows the excitation energy is

transferred to both chlorophyll *a* and *b* and the energy transfer processes are also via the S_2 states of chlorophyll *b* and chlorophyll *a* at around 580 nm and 605 nm respectively. The bleaching and stimulated emission at around 650 nm and 680 nm due to direct excitation of chlorophyll *b* and *a* respectively indicates the energy transfer from carotenoids to both chlorophylls. The energy transfer processes from chlorophyll *b* (652 nm) to unbound chlorophyll *a* (672 nm) and bound chlorophyll *a* (683 nm) are represented in the 817 fs spectrum. The 7.71 ps processes exhibit two energy transfer processes, one is from chlorophyll *b* around 655 nm to chlorophyll *a* at around 683 nm and the other one is from unbound chlorophyll *a* (672 nm) to chlorophyll *a* [5.5]. From the 817 fs and 7.71 ps DADS, two chlorophyll *b* pools are shown and resolved as the peaks at 652 nm and 655 nm. We will label them as marked as Chlb⁺ and Chlb⁻, respectively. The chlorophyll *b* pools are also observed by Connelly [5.7, 5.15]. At a time constant of 69.1 ps, the bleaching and stimulated emission of chlorophyll *b* at around 650 nm disappears and only the signal due to the absorption of chlorophyll *a* is shown in the DADS.

Figure 5.6: The kinetics curves for wild type LHC II upon excitation with 50 fs pulses at 500 nm. Scatter: raw data; Solid curve: the fit.

The kinetics traces for transient absorption spectra of wild type LHC II upon excitation with 500 nm pulses at the wavelengths 652 nm, 672 nm, and 681 nm are plotted in Figure 5.4. The signal due to the bleaching and stimulated emission of chlorophyll *b* at 652 nm has a small strength, exhibits a fast rise on the sub-picosecond time scale and decays to 0 in a few of picoseconds. The other two kinetics curves of wild type LHC II at 672 nm and 681 nm are similar with that of native LHC II. The 672 nm signal starts to decay and the 681 nm signal is still rising at around the delay time of 5 ps. Both signals have a fast rise with slower phases within around the 1 ps delay time.

Figure 5.7: DADS obtained by global analysis for transient absorption spectra of wild type LHC II upon excitation with 50 fs pulses at 500 nm.

Similar with native LHC II, a five-component global analysis was used to obtain the fit of transient absorption kinetics for wild type LHC II at all wavelengths. Figure 5.6 and 5.7 illustrate the satisfactory fit of three kinetics traces curves and the calculated DADS. The time constants are listed: 125 fs, 779 fs, 6.49 ps, 36.7 ps, and 2.16 ns. Same reason with

native LHC II, the accuracy of the 2.16 ns component indicating the bleaching and stimulated emission signals due to the Q_y band of chlorophyll *a* is not high. The energy transfer processes of carotenoid to chlorophyll *a* and *b* occur at the delay time of 125 fs, and the S_2 states of chlorophyll *a* at around 605 nm and chlorophyll *b* at around 580 nm also take apart in these energy transfer processes. The 779 fs spectrum clearly exhibits the energy transfer from the Chlb` pool at around 652 nm to chlorophyll *a* with the bleaching and stimulated emission at around 681 nm and 672 nm. From the 6.49 ps DADS, the energy transfer processes from the Chlb`` pool at around 656 nm to chlorophyll *a* at around 681 nm and from unbound chlorophyll *a* with absorption at 672 nm to the 681 nm chlorophyll *a* are easily observed. Only the decay process of the bleaching and stimulated emission of chlorophyll *a* at around 681 nm can be found in the 36.7 ps process. Compared to the results of native LHC II, The energy transfer processes of carotenoid $\rightarrow$ chlorophyll and chlorophyll *b* $\rightarrow$ chlorophyll *a* and unbound chlorophyll *a* $\rightarrow$ chlorophyll *a* in wild type LHC II are faster than these processes in native LHC II.

Figure 5.8: The kinetics curves for wild type LHC II upon excitation with 50 fs pulses at 500 nm. Scatter: raw data; Solid curve: the fit.

Figure 5.8 shows the typical kinetics curves for the H120L mutant LHC II. Compared with the kinetics traces of native and wild type LHC II, a very clear feature is that the strength of the signal due to the bleaching and stimulated emission of chlorophyll *b* is quite low, as shown in the 656 nm curve. The 672 nm and 682 nm kinetics traces are very similar to the results of the native and wild type LHC II. These features of the H120L LHC II indicate that the energy transfer from carotenoid to chlorophyll *b* has been affected by the mutant part with no observable effects of the energy transfer from carotenoid to chlorophyll *a*.

Figure 5.9: DADS obtained by global analysis for transient absorption spectra of H120L mutant LHC II.

The DADS obtained by global analysis for transient absorption spectra of H120L mutant LHC II is plotted in Figure 5.9. The analysis contains five decay components: 133 fs, 965 fs, 6.8 ps, 40.9 ps and 2.44 ns. From Figure 5.8, we can see a good fit has been achieved. Due to the 800 ps limit of the delay time, the 2.44 ns DADS may be not quite precise, and shows the bleaching and stimulated emission signals due to the Q_y band of chlorophyll *a*. The energy

transfer processes of carotenoid to chlorophyll via the S_2 states of chlorophyll *b* and *a* centred at around 580 nm and 605 nm occur at the delay time of 133 fs, and an obvious feature of H120L mutant LHC II is that the excitation energy transfer process from carotenoid to the S_1 state of chlorophyll *b* is hard to occur. The 779 fs process only contains the excitation energy transfer from the chlorophyll *b* to the chlorophyll *a*. We can know the excitation energy is transferred from the Chlb⁺⁺ pool at around 656 nm to chlorophyll *a* at around 681 nm and from unbound chlorophyll *a* with absorption at 672 nm to the 681 nm chlorophyll *a* from the 6.8 ps DADS. The 40.9 ps spectrum shows the excited chlorophyll *a* with the absorption centred at around 681 nm is decaying to the ground state.

5.4 Discussion

The results obtained from three types of LHC II upon excitation at 500 nm shows some features of the carotenoid-to-chlorophyll energy transfer. For the native and wild type LHC II, the excitation energy absorbed by the S_2 state of carotenoid is transferred to the pools of chlorophyll *a* and *b* at around 100 fs. That should be because that the structures of native and wild type LHC II are very similar. However, for H120L mutant LHC II, most of the excitation energy will be transferred to the chlorophyll *a* pools at around 133 fs. Furthermore, all the three types of LHC II show the energy transfer process from the Chlb⁺⁺ pool to chlorophyll *a* occurs in a 0.8-1.0 ps time scale. The unbound chlorophyll *a* at around 672 nm to the 681 nm chlorophyll *a* pool energy transfer process can be observed at 6-8 ps in all the LHC II, but the Chlb⁺⁺ pool to chlorophyll *a* absorbing at around 680 nm only occurs in the native and wild type LHC II. After tens of picoseconds, only the processes due to the bleaching and stimulated emission of chlorophyll *a* decays can be observed in the three types of LHC II.

In the chlorophyll *b* absorption regions, a fast rise occurs in the absorption difference spectra for native and wild type LHC II, and the chlorophyll *b* region is considered to be due to two chlorophyll *b* pools resolved at 652 nm (Chlb⁺) and 655 nm (Chlb⁺). The rise in the signal of H120L mutant LHC II is quite small, that means most of the excitation energy is not transferred to chlorophyll *a* via chlorophyll *b*. After the excitation energy is transferred to the chlorophyll, the energy transfer between chlorophyll *b* and among chlorophyll *a* occurs in few of picoseconds.

Connelly et al have claimed that the energy transfer from carotenoid to chlorophyll *b* followed by a slower energy transfer from chlorophyll *b* to chlorophyll *a* dominate the entire carotenoid to chlorophyll energy transfer process at the time delay of 141 fs in the wild type LHC II [5.11]. However, our result of wild type LHC II shows the energy transfer from carotenoid to chlorophyll contain two processes: the main one is that the excitation energy is guided to the chlorophyll *a* via the Q_x bands of chlorophyll *a* and *b*, and only a small part of the excitation energy is transferred to chlorophyll *b*.

In our results for all the three types of LHC II, in the Q_y absorption region two chlorophyll *a* pools including the unbound chlorophyll *a* absorbing at around 672 nm and the chlorophyll *a* pool at around 682 nm are apparent. We cannot find clearly the chlorophyll *a* pool at around 679 nm which is mentioned in [5.11].

5.5 Conclusion

In our study, the carotenoid to chlorophyll energy transfer processes in native, wild type and H120L mutant LHC II were measured by ultrafast transient absorption spectrometer. A five-component global analysis was used to describe the carotenoid to chlorophyll energy transfer process.

In few hundreds fs time scale, the energy absorbed by carotenoids is transferred to both of chlorophyll *a* and *b* via the Q_x bands of chlorophyll *a* and *b* in the native and wild type LHC II. The possible reason should be that these two LHC IIs have similar structure and they are from the expression of the same genes. However, in the H120L mutant LHC II, most of the excitation energy is only transferred to chlorophyll *a* via the S_2 states of chlorophyll *a* and *b*, the leucine in the H120L mutant LHC II causes the energy absorbed by carotenoids is hard to be transferred to chlorophyll *b*.

In the following chlorophyll *b* to chlorophyll *a* energy transfer processes, all the three types of LHC IIs express similar features, as shown in the latter time components at a few of picoseconds. However, compared to the energy transfer process from chlorophyll *b* and unbound chlorophyll *a* to bound chlorophyll *a* in native LHC II (69 ps), the similar processes in wild type and H120L LHC II (35-40 ps) are faster. This case should be due to the reconstitution in the cyanobacterial.

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Chapter 6

The Dynamical Study of Excitation Energy

Transfer in Plant Photosystem I

6.1 Introduction

Photosystem I (PS I) complex is a key part of the photosynthetic process in all oxygenic photosynthetic organisms, such as cyanobacteria, algae and plants. It utilizes absorbed photon energy to drive the oxidation of plastocyanine, on its donor side, and the reduction of ferredoxin, on its acceptor side. Biochemically, PS I is a large multi-subunit protein complex binding a large number of pigments and cofactors [6.1, 6.2].

The study of the overall structure of PS I in higher plants or bacteria has attracted continuing interest. In 1980s, Boekema et al used electron microscopy and computer image analysis technique to investigate the structure of PSI isolated from spinach and cyanobacterial and discussed the arrangement of the subunits [6.3, 6.4, 6.5]. Recently, the atomic-resolution crystal structures of plant and cyanobacterial PS I have been provided by different groups [6.6, 6.7, 6.8]. The so-called core complex binds the majority of light-absorbing pigments (core antenna), the reaction centre P700 and the electron-transport chain. Through these previous researches, it is clear that PS I isolated from cyanobacterial is organized as a trimer with three monomeric units, whereas the plant PS I is only found as a monomer. One monomeric unit of cyanobacterial PS I comprises 12 proteins subunits binding 96 chlorophylls, 2 phylloquinones, 3 Fe_4S_4 clusters, 22 carotenoids, 4 lipids [6.6], while the

plant PS I consists of 17 protein subunits coordinating more than 178 cofactors including 168 chlorophylls, 2 phyloquinones, 3 Fe₄S₄ clusters, some carotenoids and lipids [6.8].

Furthermore, in green algae and higher plants the core complex is surrounded by additional membrane pigment-binding complexes (Light-Harvesting Complex I), containing chlorophyll *a*, chlorophyll *b* and xanthophylls and acting as peripheral antenna units. PS I of higher plants binds four LHC Is and up to nine complexes are found in green algae [6.9]. Remarkably, despite of the structural complexity and great number of absorbing pigments, PS I performs photochemical charge separation with quantum yield of near unity. A crucial prerequisite of this high efficiency is that absorbed light energy from any of the antenna pigment is transferred extremely fast to the reaction centre before it can be lost via natural de-excitation processes.

In order to gain detailed information about the relationship among the components of PS I, various spectroscopic techniques have been applied to investigate the dynamical process in PS I. The bulk of the antenna chlorophylls surrounding the reaction centre P700 absorb at wavelengths in the range of 660 to 680 nm [6.10, 6.11, 6.12]. A unique spectroscopic feature of PS I, especially from plants and green algae, is the existence of so-called “red” chlorophylls, absorbing light at wavelengths longer than the reaction centre. This necessitates energetically unfavourable uphill energy transfer to the reaction centre and poses the question how the “red” chlorophylls affect the efficiency a dynamics of charge separation. In plants these red chlorophyll molecules in PS I can absorb at wavelengths of 708 nm, 712 nm, 725 nm and 730 nm [6.11, 6.12, 6.13], but the number and positions depend on species. All or some of the red chlorophylls are located at the periphery of the reaction centre of PS I [6.14]. The excitation energy transfer and trapping processes in the cyanobacterial PS I have been studied by Savikhin and others [6.1, 6.15]. However, as mentioned in the above section, the

PS I is organised as a trimeric unit in the cyanobacterial and exists as a monomer in eukaryotic algae and higher plants [6.16, 6.17].

In this chapter, femtosecond transient absorption spectroscopic technique was applied to study the energy transfer process in the plant PS I. The temporal width of pump pulse is around 50 fs, and the ultrashort pulse allow us to observe the dynamical process in the plant PS I.

6.2 Materials and Methods

The plant PS I complexes used in the pump probe experiment were purified from pea and were dissolved in the buffer. Details of the purification process can be found at [6.18]. The solutions were put into a 1 mm path length flowing cell and adjusted to provide an optical density of 0.5 at the maximum absorption band of 680 nm.

Femtosecond pump-probe spectrometer used in this experiment has been described in Chapter 3.4. In this experiment, the pump beam centre wavelength was set to 650 nm, and the pump pulse energy was maintained at 5 nJ. A home-built autocorrelator was used to check the pulse duration, and the temporal width of the pump pulses is around 50 fs. The diameter of focus point was calculated to be 100 μm . To obtain the population-only dynamics, the polarization of the pump beam was set to the magic angle of 54.7° with respect to the probe beam. All pump probe measurements were performed at room temperature (25°C). Linear ground state absorption spectra were taken before and after the transient absorption measurements to make sure that no sample degradation occurred during the measurement.

6.3 Experimental Results

6.3.1 Steady-State Absorption Spectrum

Figure 6.1: The steady-state absorption spectrum of plant PS I at room temperature and the spectrum of pump beam used in the experimental.

The steady-state absorption spectrum of PS I and the spectrum of the excitation pulses used in the pump probe experiment are shown in Figure 6.1. The main absorption peak at around 680 nm is due to the Q_y absorption transition of bulk antenna pigments. The pump pulse at 650 nm with 30 nm bandwidth was used to excite preferentially the chlorophyll *b* containing peripheral antenna complexes. The maximum pulse energy was limited to 5 nJ to avoid damage of the PS I and distortion of the kinetics by multiphoton events (singlet-singlet annihilation).

6.3.2 Transient Absorption Difference Spectra

Figure 6.2: Upper: The contour map of transient absorption spectra for plant PS I upon excitation with 50 fs pulse at 650 nm as a function of time delay and wavelength. Lower: Transient absorption profiles for PS I at the time delays: 100 fs, 1 ps, 10 ps and 200 ps.

The absorption difference spectra versus time delay and probe wavelength for plant PS I excited at 650 nm were plotted in Figure 6.2. All the absorption difference profiles from 630 nm to 750 nm were recorded. The time delay was scanned from -2 to 200 ps. The Q_y band of chlorophyll *b* in plant PS I was excited. The signals due to the bleaching and stimulated emission of bulk antenna pigments are dominant in the transient absorption spectra. The maximum pulse energy was limited to 5 nJ to prevent the PS I from photo-damage. Before

and after experiment, the linear absorption spectra were taken to make sure that the PS I sample has no change during the measurement. Due to the temporal chirp of the probe light, the light components of different wavelengths reach the sample at different time delays. It can be shown in the result by the coherent artifact. A polynomial equation up to second order (wavelength as a function of time delay) was used to correct the dispersion effect. The detail can be found in Section 3.5.

Transient absorption profiles for PS I at four different delay times of 100 fs, 1 ps, 10 ps and 200 ps are illustrated in the lower part of Figure 6.2. A few of the dynamical processes can be directly observed. Transient absorption spectrum at 100 fs exhibits a small negative peak at 654 nm and a big negative peak at 685 nm with a shoulder at 672 nm. These signals should result from the peripheral antenna consisting of chlorophyll *a* and *b*. The peak at 654 nm are due to the absorption of chlorophyll *b* and the big peak at 685 nm results from A_0 absorption band of PS I due to the absorption of the charge transfer state ($P700^+$) [6.2] while the shoulder at 666 nm is the result of the energy acceptance of chlorophyll *a*. At the delay time of 1.0 ps, the negative signal at 654 nm and the shoulder signal at 666 nm disappear and the signal at 685 nm increase in the transient absorption spectrum. These changes describe the energy transfer from chlorophyll *b* and *a* to the A_0 absorption band of PS I. A broad negative peak at 685 nm with a shoulder at around 700 nm can be observed in the transient absorption spectrum of 10 ps. The shoulder at round 700 nm result from the absorption of reaction centre of PS I ($P700$). From this profile, we can know an energy transfer process from bulk antenna to the reaction centre ($P700$) occurs at the time delay of 10 ps.

6.3.3 Global Analysis

A global analysis with five lifetime components was applied to the absorption difference profiles and the five DADS components were obtained: 150 fs, 1.93 ps, 10.9 ps, 67.5 ps and

2.47 ns. The shapes of these DADS components are similar with the results for cyanobacterial PS I published in [6.15], but the lifetimes for plant PS I in our experiment are longer than the time constants of 0.43 ps, 2 ps, 6.5 ps, 24 ps and 1.08 ns for cyanobacterial PS I.

Figure 6.3: The DADS from global analysis of transient absorption difference spectra for plant PS I excited at 650 nm.

As shown in Figure 6.3, the DADS component with the shortest time decay of 150 fs indicates the energy transfer process from chlorophyll *b* at 654 nm and the chlorophyll *a* pool at 666 nm to the A_0 absorption band at around 685 nm. At the same time, a small part of the energy trapped by chlorophyll *a* and *b* is also transferred to the A_0^- absorption band corresponding to the pool of chlorophyll *a* absorbing at around 690 nm and the reaction center P700 at around 700 nm. The most ‘red’ chlorophyll at around 750 nm [6.2] is also

receiving a little of the trapped energy, as shown in the DADS spectrum of 150 fs. The ‘red’ chlorophylls which are from peripheral antenna have been reported by Shubin’s group [6.13]. The 1.93 ps DADS component is assigned to the effective energy trapping by the reaction center P700. In the 1.93 ps process, the excitation energy is also transferred to the red chlorophyll *a* molecules absorbing at around 715 nm, 730 nm and 750 nm from the chlorophyll *a* pool at around 680 nm. The peak at around 715 nm is also due to the peripheral antenna [6.12]. The 10.9 ps DADS mainly exhibits the amplitude of the bleaching and stimulation emission signal due to the A_0 absorption band fast decay. The positive signals at around 650 nm and 720 nm should be due to the excited state absorption of bulk pigment antenna. The long-time decay kinetics in the A_0 absorption band at around 685 nm and the red portion of the bulk antenna at above 700 nm are dominated by the 67.5 ps DADS and clearly observed in Figure 6.2.

6.3.4 Annihilation

In order to ensure the measurements of transient absorption spectra were performed under annihilation-free condition, the power dependence of absorption difference spectra in plant PS I was studied. A repeat experiment by using individual pump pulse energy 2.5 nJ was also carried out. A five-component global analysis was used to calculate the DADS resulting from transient absorption spectra taken at the 2.5 nJ pump pulse energy, and the five time constants are 108 fs, 1.96 ps, 10.9 ps, 71 ps, and 2.52 ns. These lifetimes are almost the same with the DADS shown in Figure 6.3. Compared to the 5 nJ DADS, the shapes of all the components of 2.5 nJ DADS have no observable changes, as illustrated in Figure 6.4. Thus, we believe that our experiments were carried out under annihilation-free condition.

Figure 6.4: The DADS resulting from transient absorption spectra excited with the individual pulse energy 2.5 nJ.

6.4 Discussion

In our experiment, the energy transfer process from bulk chlorophylls to the Q_y absorption spectral range (660-693 nm), the reaction center P700, and some red chlorophylls absorbing at around 715 nm, 730 nm and 750 nm is exhibited in the two shortest DADS components. The 150 fs DADS is assigned to downhill spectral equilibration between bulk chlorophyll. This time constant is in agreement with the 200 fs result reported in [6.19]. In the 1.93 ps process, the energy trapping by P700 occurs and the excitation energy is also transferred to

the red chlorophyll *a* molecules absorbing at around 715 nm, 730 nm and 750 nm from the chlorophyll *a* pool at around 682 nm.

Figure 6.5: The kinetics traces and the fit probed at 682 nm, 715 nm, 730 nm and 750 nm and excited at 650 nm.

The kinetics traces due to the photobleaching and stimulated emission of the red chlorophyll absorbing at 715 nm, 730 nm and 750 nm and bulk pigments at 682 nm are shown in Figure 6.5. With five lifetimes 150 fs, 1.93 ps, 10.9 ps, 67.5 ps and 2.47 ns, the agreement between the experimental data and the fit is considerably good. From the kinetics traces illustrated in Figure 6.5, after the initial rise of the 682 nm component, the subsequent decay is matched with the rise of the signals due to the “red” chlorophyll. We draw the one possible conclusion here that the excitation energy is directly transferred to the “red” chlorophyll.

Compared with the results of Savikhin of the cyanobacterial PS I, two new red chlorophyll pools absorbing at 730 nm and 750 nm have been discovered in our experiment. The lifetimes

of the energy transfer in plant PS I are longer, compared to the cyanobacterial PS I system, because the “red” chlorophylls in plant PS I slow down the energy transfer dynamics.

6.5 Conclusion

Femtosecond transient absorption difference spectrometer was used to study the energy transfer process in plant PS I excited at 650 nm. The absorption difference spectra results from the bulk antenna pigments and red chlorophylls are dominant in the total transient absorption spectra. A global analysis with five time constants 150 fs, 1.93 ps, 10.9 ps, 67.5 ps and 2.47 ns was obtained to illustrate the transient absorption spectra and obtain the DADS. The 150 fs DADS shows that the excitation energy is transferred from the blue side of bulk antenna pigments to bulk chlorophylls and red chlorophyll. The red chlorophyll pools absorbing at 715 nm, 730 nm and 750 nm can be observed from the 1.93 ps DADS, and the accepted energy is from the bulk antenna pigments absorbing at around 682 nm. The energy trapping by P700 also occurs at 1.93 ps. In the 10.9 ps process, only the signal due to the bleaching and stimulation emission of the A_0 absorption band fast decay and a little of energy is still being transferred to the deep red chlorophyll at above 715 nm. The 67.5 ps spectrum exhibits the long-time decay of the signals due to the A_0 absorption band at around 685 nm and the red portion of the bulk antenna at above 700 nm. Only the very slow decay of the bleaching and stimulated emission of bulk antenna pigments can be observed in the 2.47 ns process. Compared with the cyanobacterial PS I, the energy transfer dynamical processes are slowed down by the “red” chlorophyll in plant PS I.

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Summary and Future Work

In this thesis, ultrafast carbonyl stretching vibration dynamics in $\text{Mn}_2(\text{CO})_{10}$ has been explored by ultrafast infrared pump probe spectroscopy in Chapter 2. Then in order to study the dynamics of photosynthetic systems in the photosynthetic process, a new non-collinear pump-probe spectrometer has been designed and built. Afterward we use this new femtosecond transient absorption spectrometer to investigate the energy transfer process from the Soret band to the Q_y band of chlorophyll *a* and *b*. An interesting phenomenon, the lifetime of the energy transfer from the Soret band to the Q_y band of chlorophyll *a* is shorter than that of chlorophyll *b* while the energy gap between the S_n state and the S_1 state of chlorophyll *a* is larger than that of chlorophyll *b*, has been described.

In the following chapters, the new non-collinear pump probe spectrometer was used to study the carotenoid to chlorophyll energy transfer dynamics in three types of LHC II and plant PS I. For native and wild type LHC II, the excitation energy absorbed by carotenoids is transferred to both of chlorophyll *a* and *b* via the Q_x bands of chlorophyll *b* and *a* in a short time scale. However, in H120L mutant LHC II the energy transfer mainly occurs between carotenoid and chlorophyll *a*. Through the global analysis of transient absorption spectra obtained from the pump probe measurements for plant PS I, the energy transfer dynamics in PS I has been discussed and some red chlorophyll pools at around 715 nm, 730 nm and 750 nm were discovered.

In the future, because of the complexity of the energy transfer processes in photosynthetic systems, ultrafast two-dimensional (2D) spectroscopy even three-dimensional (3D) spectroscopy should be applied to study the dynamics in photosynthetic systems and more valuable insights will be provided. Due to the energy levels or excitons in photosynthetic systems are coupled to each other and the direct evidence of coupling only can be gained by

the 2D spectroscopy, based on a pulse shaper, we will build a 2D spectrometer and try to obtain the farther information in photosynthetic systems.