

## ARTICLE

## Transient Absorption of the Chlorophyll a in Ethanol

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(Dated: Received on December 18, 2004; Accepted on April 22, 2005)

The excited state of Chlorophyll a is investigated by femtosecond transient absorption. The transient absorption spectra of Q band and  $B_y$  band of Chlorophyll a in ethanol have been observed. The fast kinetics of Chlorophyll a which exhibit two ultrafast components were also measured. The one is assigned to transient absorption of the inhomogeneously broadened ground state absorption spectrum, while the other is the response of the solvent to the change of the electron configuration in the excited state due to solvation dynamics of the polar solvent molecules. To understand the anisotropy of Chlorophyll a in ethanol, the anisotropy profile was also performed by 405 nm excitation and found that the anisotropy profile is 0.143. The possible combination of  $\theta_{da}$ ,  $\theta_{db}$  and  $\eta$  at excitation of  $B_y$  band has been simulated.

**Key words:** Femtosecond, Chlorophyll a, Transient absorption

## I. INTRODUCTION

Chlorophyll molecules are the green pigments that can be found in the two kinds of photosynthetic proteins—light harvesting complexes and reaction centers. The function of light harvesting complexes is to collect the sunlight and convert it to stable chemical energy in reaction centers. Some investigations on dynamics in the proteins showed that the timescale of dynamics was from seconds to femtoseconds. Various spectroscopic techniques, including transient absorption spectroscopy and time-resolved fluorescence techniques [1-3], have been used to investigate the excited-state dynamics of the function of these proteins. In addition, the energy transfer and electron transfer in photosystem [4-9] have been studied by means of femtosecond transient absorption spectroscopy and transient hole burning methods. Chlorophyll a (Chl a), as shown in Fig.1, is the principal photosynthetic pigment in algae and higher plants, which has been found in the light-harvesting complex I (LHC I) and light-harvesting complex II (LHC II) residing in the higher plants. In addition, it also exists in photochemical reaction centers of both photosystem I (PS I) and photosystem II (PS II). Figure 2 shows the absorption, emission and CD spectra of Chl a in ethanol. The absorption spectrum of Chl a has peaks of the Q band at 663, 618, 580, and 535 nm. The proposal to explain the peaks of Q is that the asymmetry destroys the in-plane degeneracy resulting in a profound effect on the electronic absorption spectrum [10,11]. The typical Q transitions of symmetrical porphyrins are split into  $x$  and  $y$  components. The CD spectrum of the Chl a in the wavelength range 400–700 nm indicates that at least three transitions exist in the Chl a. Despite the transient absorption spectra and anisotropy profiles of Chl a in pyridine

with an  $Q_y$  band excitation have been reported [12], the assignment of the other Q band of Chl a is also still ambiguous. On the other hand, so far as we are concerned, no transient absorption spectra and anisotropy of higher excited state  $B_y$  band have been investigated.

The purpose of this work is to investigate transient dynamics of excited states of Chl a in ethanol solution and present a molecular mechanism of the dynamics of this system. For this purpose, we will employ the transient absorption technique to observe transient dynamics of the Q band and  $B_y$  bands of Chl a in ethanol solution. With excitations at 405 nm (B band) and probing at Q band (665, 684 nm), we anticipate that non-radiative transition may be better takes place between the B and Q bands through internal conversion (IC).

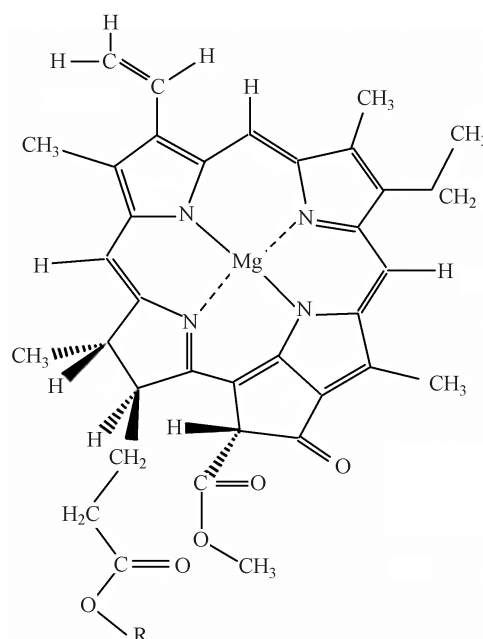


FIG. 1 Molecular structure of Chl a

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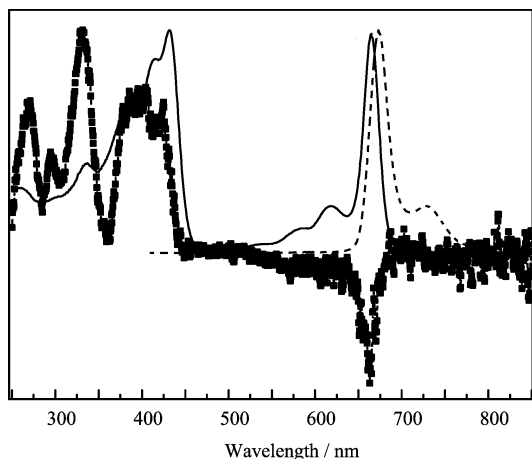


FIG. 2 The of Chl a in ethanol. The solid line(-), broken line(- -), and circles(●●●) represent steady-state absorption, emission and CD spectra respectively.

## II. MATERIALS AND METHODS

Chl a was purchased from Sigma Chemical Co. (catalogue no. C-6144) and used without further purification. In this study, Chl a was dissolved in pure ethanol solution with a concentration 40–100  $\mu\text{mol/L}$ . In order to prevent the sample from being decomposed under exposure to lights, all the experiments were processed in the dark.

A femtosecond two-color transient absorption setup was used in the time-resolved experiments. As the experimental setup for the time-resolved pump and probe femtosecond measurements has been described in previous paper [13,14], only a brief description is given here. A continuous-wave argon-ion laser (Innova 400, Coherent, Inc.) pumped Ti:Sapphire laser (Mirra 900, Coherent, Inc.). The seed pulses from the oscillator were amplified by amplifier (RegA 9000, Coherent, Inc.). The amplified pulses were used to pump an optical parametric amplifier (OPA 9400, Coherent, Inc.). The repetition rate in this experiment was operated at 20 kHz. The white-light continuum probe pulses (460–1200 nm,  $\sim 1$  nJ) was generated from OPA. The transient absorption technique was applied with excitations at absorption bands 580, 620 and 405 nm. The excitation optical density of 580 and 620 nm at the sample position was about  $4 \times 10^{14}$  photons/pulse  $\text{cm}^2$  with a pulse width 100 fs. The laser pulse width at 405 nm was about 200 fs. To monitor the rotation free isotropic decays, transient absorption kinetics were measured at the conditions of “magic angle”: excitation and probe are linearly polarized at  $54.7^\circ$  one respect to the other. A half-wave plate and a polarizer determined the angle. Measurements were performed at room temperature, unless otherwise specified.

## III. RESULTS

To understand the ultrafast mechanism taking place in Chl a within the picosecond region, we study excita-

tion dependence of transient absorption of Chl a. The signal  $\Delta A$  is defined as the difference absorption with and without the pump laser beam. The positive signals represent the contributions of induced absorption and the negative signals indicate the ground state bleaching and/or the stimulated emission.

To examine the kinetic components in more detail, we measure the temporal evolution of Chl a in ethanol. Figure 3 shows the transient absorption spectra measured at several delay times with various excitation wavelengths. The transient absorption spectra exhibit an intense ground state bleaching/stimulated emission (GB/SE) band at 664 nm, two broad induced absorption bands extend to both the red and blue edges of the GB/SE band. The zero-time spectrum displays the GB/SE maximum at 658 nm, which red shifts to 667 nm in the 15 ps spectrum. The overall temporal evolution of the transient spectra can be divided into two steps. During the first 100 fs the spectrum broadens significantly on both sides of the GB/SE band, and after 100 fs, the main GB/SE band gradually red shifts. The process of broaden is complete within 400 fs, and the equilibrium position is the same as above. The results are consistent with the transient absorption signals of Chl a in pyridine excited at 671 nm reported by Peter Martinsson *et al.* [12].

To qualitatively perform an analysis of the transient absorption spectra, we extract the kinetic curves of the Chl a and fit them with exponential functions. Since the transient absorption spectrum of Chl a in ethanol displays positive signals in the wavelength regions 400–630, 695–715 and 745–800 nm, the induce absorption mechanism is dominant in this wavelength region. Thus, we select the probe wavelength at the emission band with various excitations wavelength. The kinetics curve excited at 620 nm and probed at 663 nm exhibits a decay profile with a time constant 558 fs; while the kinetic curve excited at 620 nm and probed at 675 nm shows one rising and one decay components with time constants 0.412 and 1.75 ps, respectively. These two kinetic curves are shown in Fig.4 A and B. The kinetic curve with excitation/probing at 580/649 nm exhibits two decays components with time constants 1.1 and 20 ps; while one rising component with time constant 2.69 s is found in 580/674 nm, as shown in Fig.4 C and D, respectively. Figure 4 E exhibits one rising component at probing at 405/665 nm with time constant 5.15 ps; while two decay components are observed at 405/684 nm with time constants 0.18 and 7.75 ps, as show in Fig.4F. The time constants mentioned above and the corresponding amplitudes are listed in Table I. One or two components analysis of the measured kinetics also suggest that a medium lifetime component about 1.7–20 ps is presented.

To study transient dynamics occurring between B and Q bands, we excite Chl a at  $B_y$  band (405 nm) and probe at fluorescence region 670 nm. From these spectra the absorption anisotropy was calculated according to [15, 16] :

$$r(t) = \frac{I_{//}(t) - I_{\perp}(t)}{I_{//}(t) + 2I_{\perp}(t)} \quad (1)$$

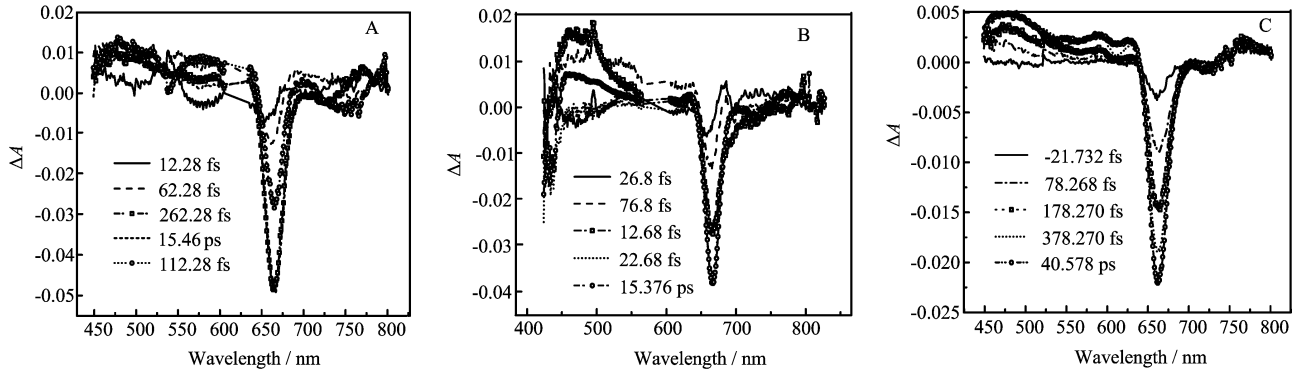


FIG. 3 The temporal evolution of transient absorption spectra of Chl a resolved in ethanol with excitations at (A) 620 nm (B) 580 nm, and (C) 405 nm.

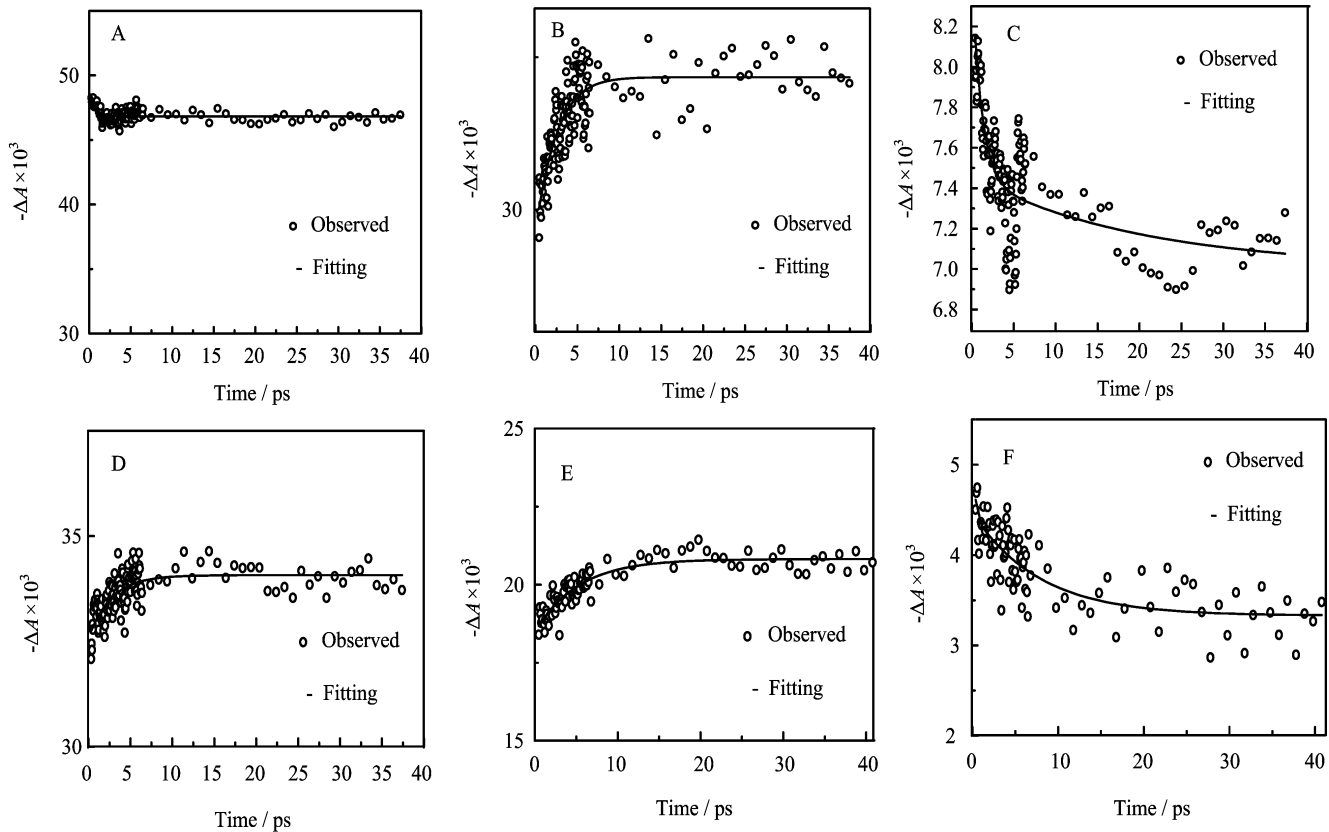


FIG. 4 The time-resolved profiles of Chl a in ethanol observed at the magic angle. (A) A fast decay component is observed upon the excitation at 620 nm and probing at 663 nm. (B) One fast rising and one decay components are observed at the excitation 620 nm and probing 675 nm. (C) Two decay components are obtained at the excitation 580 nm and probing 649 nm. (D) One rising component is found at the excitation 580 nm and probing 674 nm. (E) One rising component is observed at the excitation 405 nm and probing 665 nm. (F) Two decay components are obtained at excitation 405 nm and probing 684 nm.

where  $r(t)$  denotes the time-dependent anisotropy,  $I_{//}(t)$  and  $I_{\perp}(t)$  are the time-dependent intensities with the ordinarily parallel and perpendicular polarization configurations, respectively. Figure 5 exhibits an anisotropy profile upon the excitation at 405 nm. One can see that the anisotropy profile becomes constant about 0.143 after 200 fs within a delay time 5 ps. From anisotropy profile, we can anticipate the possible

mechanism of transient absorption [15].

#### IV. DISCUSSION

As mentioned above, the induced absorption spectrum displays a broad and featureless band. The blue excitation shows the refilling of the hole in the ground

TABLE I The optimized time constants and amplitudes of Chl a in ethanol. The time profiles are measured up to 40ps with excitations 620, 580 and 405 nm.

| (Pump/probe)/nm | $A_0\%$ | $\tau_1/\text{ps}$ | $A_1\%$ | $\tau_2/\text{ps}$ | $A_2\%$ |
|-----------------|---------|--------------------|---------|--------------------|---------|
| 620/663         | 99      | 0.558              | 1       |                    |         |
| 620/675         | 97      | 2.0                | 2       | 1.75               | 1       |
| 580/649         | 99      | 1.16               | 1       | 20                 |         |
| 580/674         | 99      | 2.69               | 1       |                    |         |
| 405/665         | 99      | 5.15               | 1       |                    |         |
| 405/684         | 95      | 0.18               | 2       | 7.75               | 3       |

state absorption due to the thermal fluctuation of the solvent. The initial bleaching moves towards the center of the band and broadens within 200 fs. This can also be seen from the kinetic of Chl a as the fast or decay component within 180–1000 fs. But it is different at the 580 nm excitation. Martinsson *et al.* [12] reported that there was only broadening of the hole but no shift when Chl a was excited at the center of  $Q_y$ , 671 nm, in pyridine. The processes that were observed are consistent with the dynamic hole burning process.

Let us now discuss the excitation wavelength dependent kinetic of the Chl a. The kinetics of the Chl a can be fitted with the exponential components [12, 17] and assigned to be various processes. The time constant 1.7–20 ps can be due to the fact that Chl a responds to the surrounding solvent and change of the electron configuration in the excited state, so called solvent dynamics of the polar solvent molecules. The similar components obtained from Chl a resolved in pyridine, from Chl b in neat pyridine [17], and from Bchl a in methanol [18] are found  $\sim 3$  ps,  $\sim 3$  ps and  $\sim 10$  ps, respectively. These different time constants obtained in various solvents are due to the polarization of solvents, which can be assigned as the dielectric relaxation of solvent.

We also find that the maximum of GB/SE band at the excitation 580 nm exhibits 9 nm shift between the initial and final delay times. It is consistent with the small amplitude of the solvation component. Martinsson has reported this shift as 10.5 nm for Chl b in neat

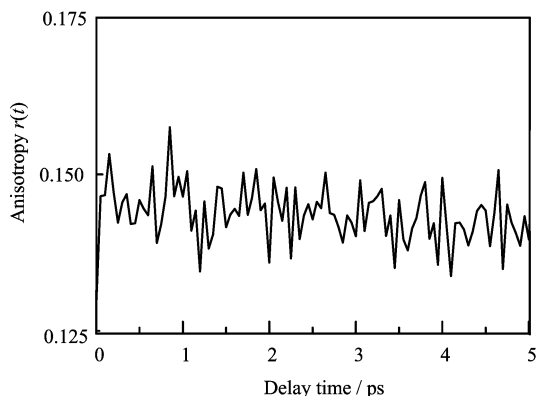


FIG. 5 The anisotropy profile at excitation 405 nm and probed at 670 nm.

pyridine and 6 nm for Chl a in pyridine. As the probe beam becomes longer, the fluorescence band may cause red shift in polar solvent. This leads the stimulated emission band red shift and, hence, GB/SEW band becomes red shift in our experiments.

Now let us discuss the anisotropy results observed in the above. The mechanism of ground state bleaching can be ignored when the probe wavelength locates at fluorescence region. According to the equation given by Shiu *et al* [15], the anisotropy can be written by

$$r(\omega, t) = \frac{\eta(1 - 3 \cos^2 \theta_{db}) - (1 - 3 \cos^2 \theta_{da})}{5(1 - \eta)} \quad (2)$$

$$\eta = K'_{S_1}(\omega, t) |\mu_b|^2 / K_{S_1}(\omega, t) |\mu_a|^2 \quad (3)$$

Here  $K_{S_1}(\omega, t)$  and  $K'_{S_1}(\omega, t)$  contain the same population dynamics associated with  $\rho_{S_1}(t)$  but different Franck-Condon factors;  $\theta_{da}$  is the relative angle of  $\mu_a$  and  $\mu_d$ .  $\mu_a$  and  $\mu_d$  represent the electronic transition dipole moment. This equation is only applied on the condition that  $\eta \neq 1$ . For the case of the excitation at 405 nm, the anisotropy is  $r(\omega, t) = 0.143$ . We simulate the possible combinations of  $\theta_{da}$ ,  $\theta_{db}$  and  $\eta$ , the details are drawn in Table II. If the induced absorption term  $\eta = K'_{S_1}(\omega, t) |\mu_b|^2$  disappears, the angle  $\theta_{da}$  should be  $40.9^\circ$ . If  $\eta \approx 100$ ,  $\theta_{db}$  should be  $40.9^\circ$  and  $\theta_{da}$  can be any value.

TABLE II The parameters of the anisotropy of 0.143 excited at 405 nm.

| $\eta$          | 0     | $1 \pm 0.02$ | 1–10   | 10–100  | >100  |
|-----------------|-------|--------------|--------|---------|-------|
| $\theta_{da}^a$ | 40.88 | 40.88        | 0–180  | 0–180   | 0–180 |
| $\theta_{db}^a$ | 0–180 | 40.88        | 44–153 | 38–40.6 | 40.88 |

<sup>a</sup> In unit of degree

## V. CONCLUSIONS

We have obtained the transient absorption spectra of Q band and  $B_y$  band of Chl a in ethanol. The absorption of excited state display strong signals in the range of our study (450–800 nm). The breadth and the maximum have shift with different excitation wavelengths. In our measurements of the time-resolved kinetics of Q band and  $B_y$  band of Chl a, one or two components are obtained. In the two fast processes, the fast components ( $< 1000$  fs) is assigned to from the transient spectral hole burning of the ground-state absorption spectrum. The 1.7–20 ps lifetime component is ascribed to be connected to the solvent relaxation.

The anisotropy profile upon the excitation at 405 nm probing at 670 nm is constant about 0.143 after 200 fs with a delay time 5 ps. We have simulated the possible combination of  $\theta_{da}$ ,  $\theta_{db}$  and  $\eta$  excitation of  $B_y$  band.

## VI. ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (NSFC No. 29825107, No.

29853001), the Knowledge Innovation Program of the Chinese Academy of Sciences (Grant: DICP K200/E3) and NKBRFSF. Support is also provided from the National Science Council of Taiwan and Academia Sinica.

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